

# SPECIFICATION

## NOVEL BICYCLONUCLEOSIDE AND OLIGONUCLEOTIDE ANALOGUE

*Subalt*  
This is a continuation-in-part application of U.S. Patent Application Serial No. 09/380,638 filed on March 9th, 1998.

### TECHNICAL FIELD

This invention relates to a novel nucleoside analogue and a novel nucleotide analogue, and more particularly, to a nucleotide analogue suitable as an antisense molecule.

### 10 BACKGROUND ART

In 1978, it was reported for the first time that an antisense molecule inhibited influenza virus infection. Since then, reports have been issued that antisense molecules inhibited the expression of oncogenes and AIDS infection. In recent years, antisense oligonucleotides have become one of the most promising pharmaceuticals, because they specifically control the expression of undesirable genes.

The antisense method is based on the idea of controlling a unidirectional flow called the central dogma, i.e., DNA → RNA → protein, by use of an antisense oligonucleotide.

When a naturally occurring oligonucleotide was applied to this method as an antisense molecule, however, it was decomposed with various nucleases in vivo, or its permeation through the cell membrane was not high. To solve these problems, numerous nucleic acid derivatives and analogues have been synthesized, and their studies have been

conducted. Examples of the synthesized products include a phosphorothioate having a sulfur atom substituting for an oxygen atom on the phosphorus atom, and a methylphosphonate having a substituting methyl group. Recently, products have  
5 been synthesized in which the phosphorus atom has also been substituted by a carbon atom, or the structure of the sugar portion has been changed, or the nucleic acid base has been modified. Any resulting derivatives or analogues, however, have not been fully satisfactory in terms of in vivo  
10 stability, ease of synthesis, and sequence specificity (the property of selectively controlling the expression of a particular gene alone).

Under these circumstances, there has been a demand for the creation of an antisense molecule which is minimally  
15 decomposed with a nuclease in vivo, binds to target messenger RNA with high affinity, has high specificity, and can thus efficiently control the expression of a particular gene.

#### DISCLOSURE OF THE INVENTION

20 The inventors of the present invention designed a nucleic acid analogue with immobilized conformation of the sugar portion in a nucleic acid, which would be useful in the antisense method. They synthesized a nucleoside analogue which will be a unit structure therefor, and  
25 confirmed that an oligonucleotide analogue prepared using it was very useful as an antisense molecule.

#### BRIEF DESCRIPTION OF THE DRAWINGS

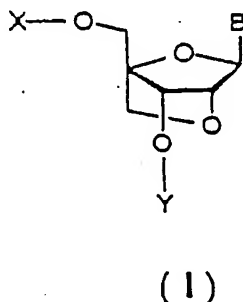
Fig. 1 is a chart showing the time course of the

ultraviolet absorption (260 nm) of a naturally occurring oligonucleotide decomposed with an exonuclease; and

Fig. 2 is a chart showing the time course of the ultraviolet absorption (260 nm) of an oligonucleotide of the present invention (X2) decomposed with an exonuclease.

Details of the present invention will now be described.

The structure of a nucleoside analogue according to the present invention is a nucleoside analogue of the following general formula (I):



where B is a pyrimidine or purine nucleic acid base, or an analogue thereof, and X and Y are identical or different, and each represent a hydrogen atom, an alkyl group, an alkenyl group, an alkynyl group, a cycloalkyl group, an aralkyl group, an aryl group, an acyl group, or a silyl group, or an amidite derivative thereof.

The alkyl group represents a straight chain or branched chain alkyl group with 1 to 20 carbon atoms. Its examples include methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, pentyl, hexyl, heptyl, octyl, nonyl and decyl.

The alkenyl group represents a straight chain or branched chain alkenyl group with 2 to 20 carbon atoms. Its examples include vinyl, allyl, butenyl, pentenyl, geranyl, and farnesyl.

- 5           The alkynyl group represents a straight chain or branched chain alkynyl group with 2 to 20 carbon atoms. Its examples include ethynyl, propynyl, and butynyl.

- The cycloalkyl group represents a cycloalkyl group with 3 to 8 carbon atoms, and includes, for example,  
10   cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Another example is a heterocyclic group in which one or more arbitrary methylene groups on the ring of the cycloalkyl group have been substituted by an oxygen atom, a sulfur atom, or an alkyl-  
15   substituted nitrogen atom. It is, for instance, a tetrahydropyranyl group.

- The aryl group refers to a monovalent substituent formed by removing one hydrogen atom from an aromatic heterocyclic group or an aromatic hydrocarbon group.  
20   Preferably, it represents a monovalent substituent formed by removing one hydrogen atom from an aromatic hydrocarbon group, and includes, for example, phenyl, tolyl, xylyl, biphenyl, naphthyl, anthryl, and phenanthryl. The carbon atom on the ring of the aryl group may be substituted by one  
25   or more of a halogen atom, a lower alkyl group, a hydroxyl group, an alkoxyl group, an amino group, a nitro group, and a trifluoromethyl group. The substituent in this case is, for example, a halogen atom, a hydroxyl group, an amino

group, an alkoxy group, or an aryloxy group.

The aralkyl group refers to an alkyl group bonded to an aryl group, and may be substituted. The aralkyl group that may be substituted represents an alkyl group bonded to an aryl group, with one or more arbitrary hydrogen atoms of the aryl group and the alkyl group being optionally substituted by the following substituents: Examples of the substituents are acyl, amino, aryl, alkyl, cycloalkyl, alkoxy, hydroxyl, nitro, and halogen.

10 The amino group need not be substituted, but the amino group when substituted includes, for example, alkylamino, arylamino, and acylamino. Examples of the alkoxy group are methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy, s-butoxy, t-butoxy, pentyloxy, hexyloxy, 15 and phenoxy. Examples of the halogen atom are fluorine, chlorine, bromine, and iodine.

The preferred examples of the aralkyl group are trityl, benzyl, phenethyl, tritylmethyl, diphenylmethyl, naphthylmethyl, and 4,4'-dimethoxytrityl (DMTr). 20 Particularly preferred is a DMTr group.

As the acyl group, acetyl, formyl, propionyl, benzoyl, and benzyloxycarbonyl can be exemplified. An example of the silyl group is a trialkylsilyl group, preferably trimethylsilyl, triethylsilyl, triisopropylsilyl, t-butyl- 25 dimethylsilyl or t-butyl-diphenylsilyl, and more preferably trimethylsilyl.

The nucleotide analogue of the present invention is an oligonucleotide or polynucleotide analogue having one or

[illegible]

5



10

group, a phosphoric acid residue, a naturally occurring nucleoside or a synthetic nucleoside bound via a phosphodiester bond, or an oligonucleotide or polynucleotide containing the nucleoside,  $n^1$ 's or  $n^2$ 's are identical or different, and each denote an integer of 0 to 50, provided that  $n^1$ 's or  $n^2$ 's are not zero at the same time, and that not all of  $n^2$ 's are zero at the same time,  $n^3$  denotes an integer of 1 to 50, provided that when  $n^1$  and/or  $n^2$  are or is 2 or more,  $B^1$  and B need not be identical, and R's need not be identical.

The pyrimidine or purine nucleic acid base in the present invention refers to thymine, uracil, cytosine, adenine, guanine, or derivatives thereof.

An analogue of pyrimidine or purine nucleic acid base in the present invention includes any modified nucleic acid bases suitable for the purpose of the present invention. In particular, the analogue of pyrimidine or purine nucleic acid base useful in the present invention includes any modified nucleic acid base which can provide an oligonucleotide analogue suitable as an antisense oligonucleotide of the present invention when the modified nucleic acid base is introduced in the nucleotide analogues of the present invention as a nucleic acid base moiety.

An analogue of pyrimidine or purine nucleic acid base which is useful in the present invention may be selected from the prior art or may be easily prepared by a person skilled in the art. Examples of the analogue of pyrimidine

or purine nucleic acid base are described, for example, in the following references. However, it should not be construed that the analogue of the present invention is limited to those disclosed in the references.

5           Incidentally, all of the references listed below are incorporated in the present application by reference.

(1) I. Luyten and P. Herdewijin, Eur. J. Med. Chem. 33, 515-576 (1998)

(2) Albert L. Lehninger et al. "Principles of Biochemistry" 10 Second Edition, (1993) page 327, Fig. 12-5

(3) Stanley T. Crooke et al., "Antisense Research and Applications" (1993), page 277, Fig. 2 and page 282, Fig.3

(4) B.A. Schweitzer and E.T. Kool, J. Am. Chem. Soc., 117, 1863-1872 (1995)

15 (5) S.O. Doronia et al., Chem. Soc. Rev., (1997), 63-71

(6) N.C. Chaudhuri, et al., Synlett, 1997, 341-347

(7) T. E. Lehmann, et al., Chim. Acta, 80, 2002-2022 (1997)

(8) C.A. Stein et al., Science, 261, 1004-1012 (1993)

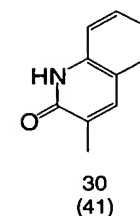
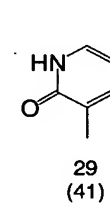
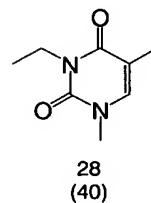
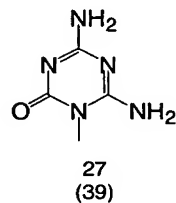
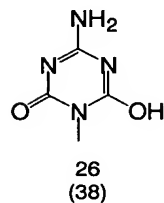
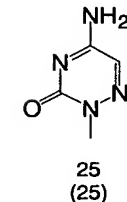
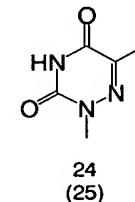
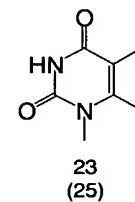
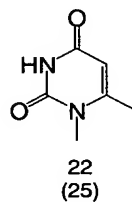
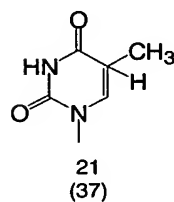
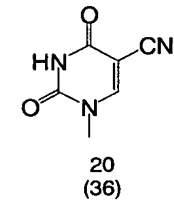
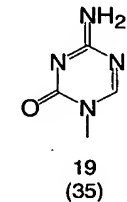
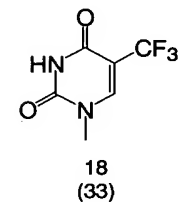
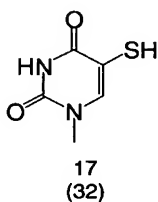
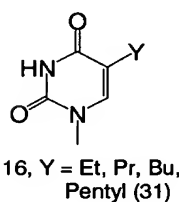
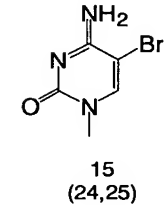
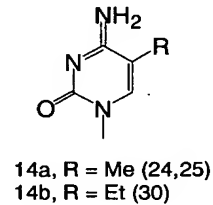
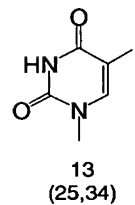
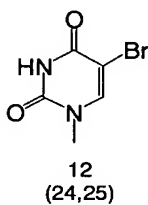
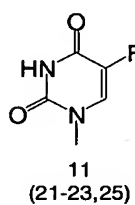
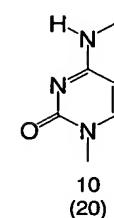
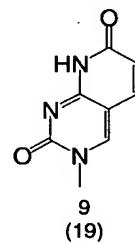
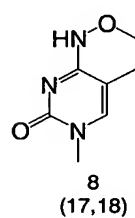
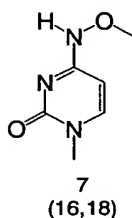
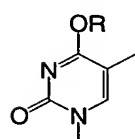
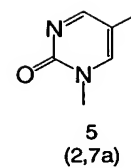
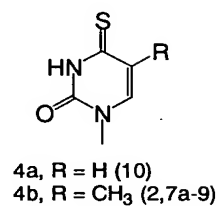
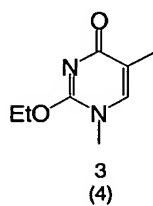
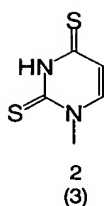
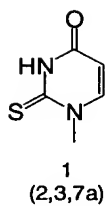
(9) E. Uhlmann et al., Chem. Rev., 90, 543-584 (1990)

20           Some examples of modified pyrimidine and purine nucleic acid base are listed below. However, they are listed only for the purpose of showing examples and therefore should not be construed as limiting the present invention.

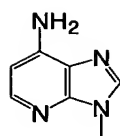
(Examples of Modified pyrimidine or purine nucleic acid  
25 base)



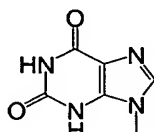
# Pyrimidine modifications



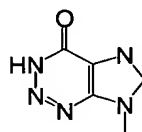
# Purine modifications



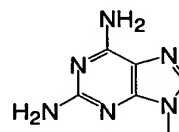
31  
(42)



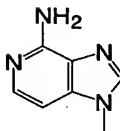
32  
(43)



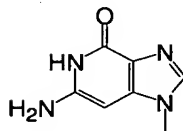
33  
(44)



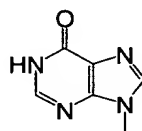
34  
(45,46)



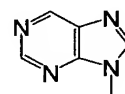
35  
(42,47)



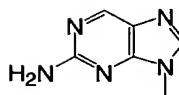
36  
(48,49)



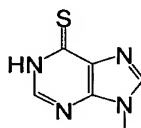
37  
(50,51)



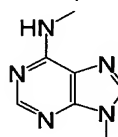
38  
(7a,43)



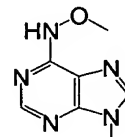
39  
(7a,52,53)



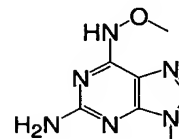
40  
(7a,9)



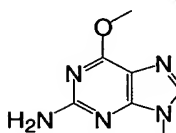
41  
(20,54)



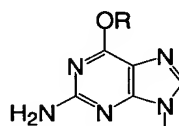
42  
(55,56)



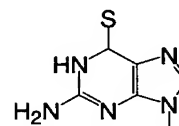
47  
(58)



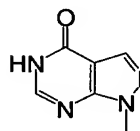
48  
(58)



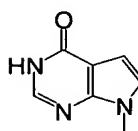
49a, R = H (59)  
49b, R = Me (60)



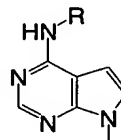
50a, R = H (59)  
50b, R = Me (60)



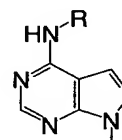
51  
(61)



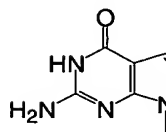
52  
(61)



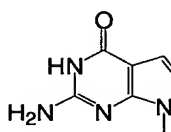
53  
(62,65)



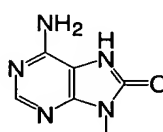
54  
(64)



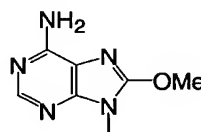
51  
(61)



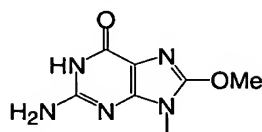
52  
(61)



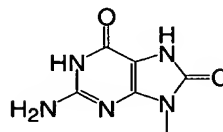
53  
(62,65)



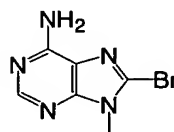
54  
(64)



55  
(64)



56  
(63,64)

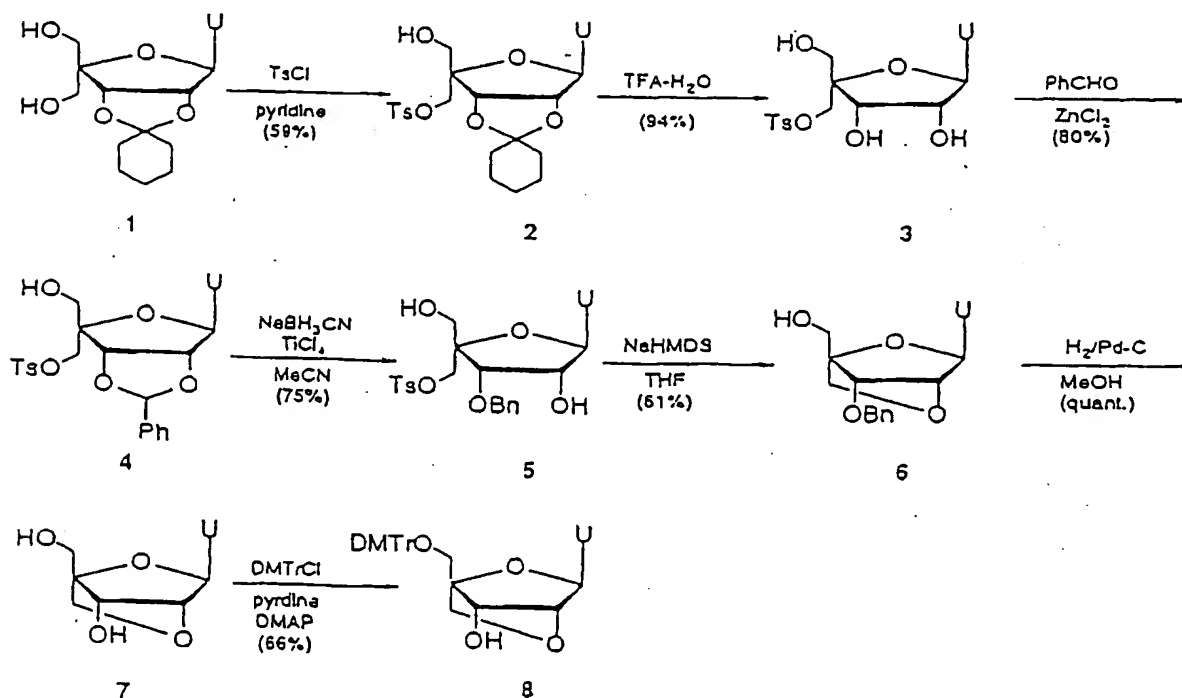


57  
(65)

The nucleoside analogue and nucleotide analogue of the present invention can be synthesized in the manner described below.

In the following description, uracil is mainly used as a base, but other purine nucleic acid bases, pyrimidine nucleic acid bases and analogues thereof can also be used similarly.

(1) Synthesis of nucleoside analogue

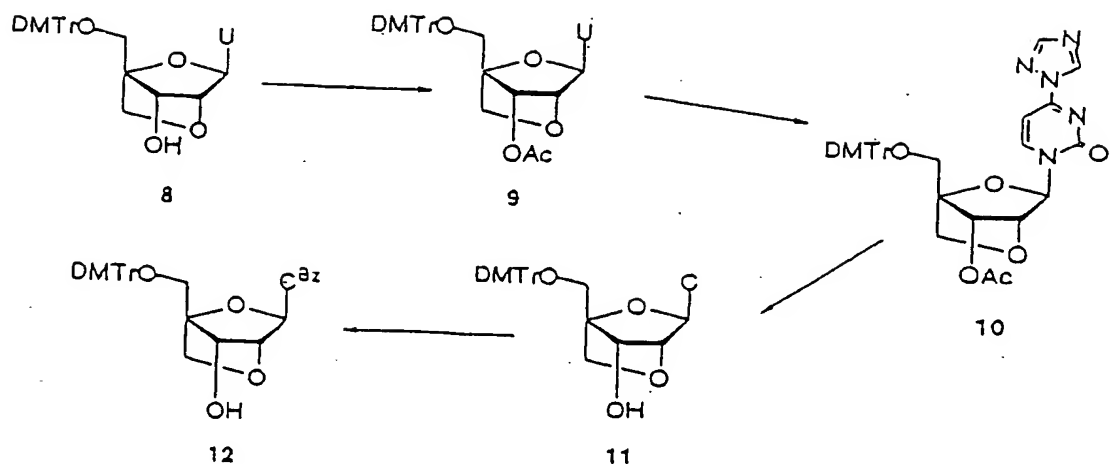


Compound 1, synthesized from uridine in accordance with the literature [1) J.A. Secrist et al., J. Am. Chem. Soc., 101, 1554 (1979); 2) G.H. Jones et al., J. Org. Chem., 44, 1309 (1979)], was treated with tosyl chloride (TsCl) to tosylate only one of the two primary alcohols, leading to Compound 2. Compound 2 was acid hydrolyzed into a triol compound 3. Compound 3 was condensed with benzaldehyde in

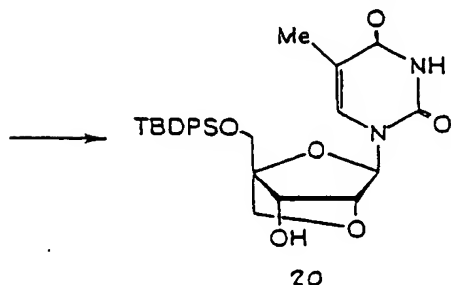
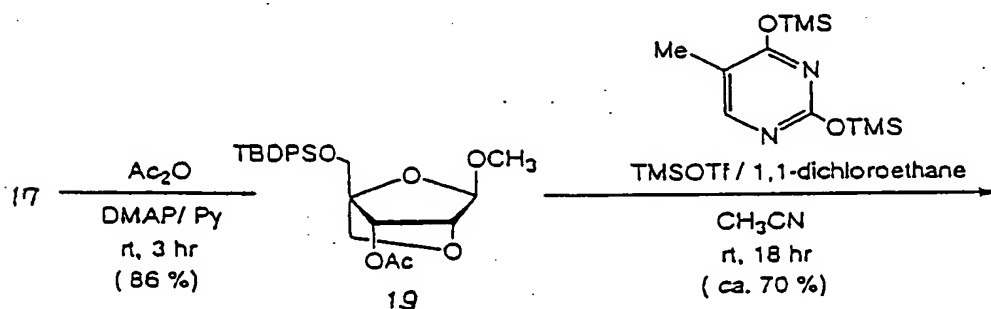
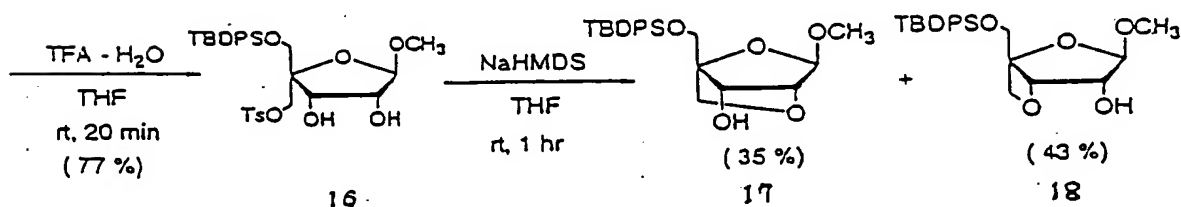
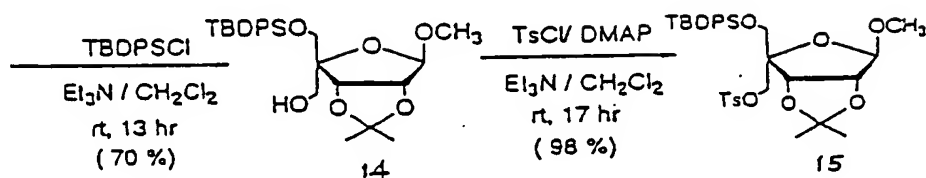
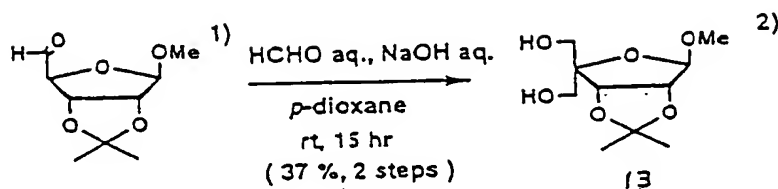
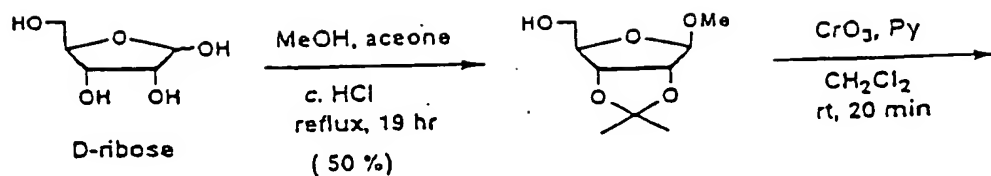
the presence of an acid catalyst to form a benzylidene compound 4. Compound 4 was reduced with sodium cyanoborohydride ( $\text{NaBH}_3\text{CN}$ ) in the presence of titanium tetrachloride ( $\text{TiCl}_4$ ) to obtain Compound 5. This compound 5 was reacted with sodium hexamethyldisilazide ( $\text{NaHMDS}$ ) in tetrahydrofuran (THF) to obtain a bicyclo compound 6 (Compound I: B = uracil (U), X = H, Y = benzyl). When Compound 6 was catalytically reduced in the presence of a palladium carbon catalyst, a diol compound 7 (Compound (I): B = U, X = Y = H) was obtained. Further treatment of Compound 7 with 4,4'-dimethoxytrityl chloride (DMTrCl) gave a trityl compound 8 (Compound I: B = U, X = DMTr, Y = H). Compounds 6, 7 and 8 can be used as starting materials for various compounds I.

15           Compounds (I) having various nucleic acid bases, whether natural or nonnatural, other than uridine, can be synthesized by any of the following three methods:

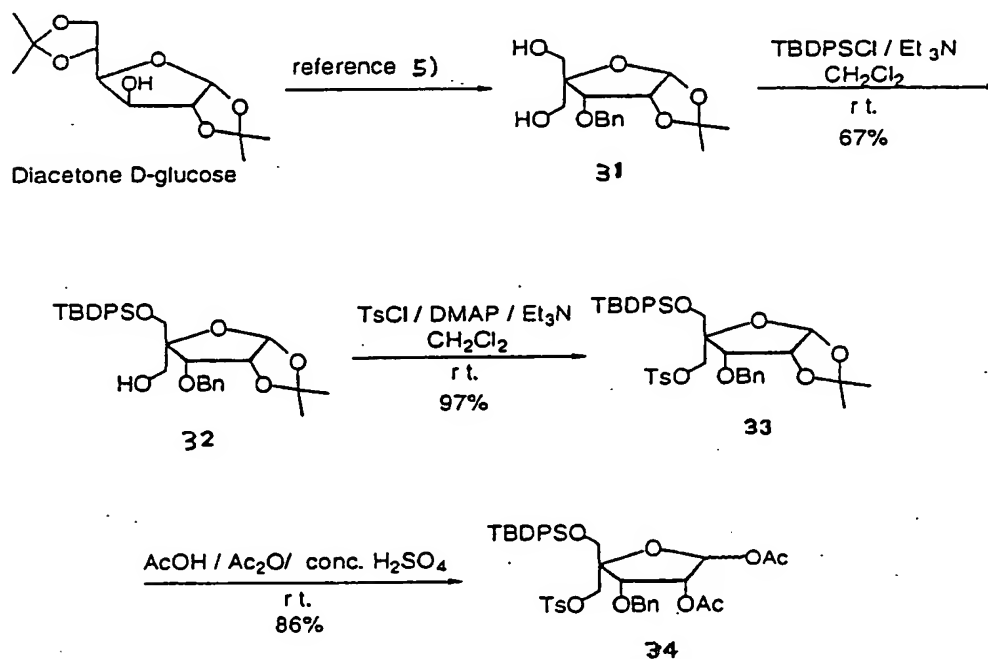
          The first method is conversion from Compound 8. That is, Compound 8 is acetylated into Compound 9, and then 20 reacted with 1,2,4-triazole to form Compound 10. Hydrolysis of this compound gave Compound 11 (Compound (I): B = cytosine (C), X = DMTr, Y = H). Compound 12 (Compound (I): B = benzoylcytosine ( $\text{C}^{\text{Bz}}$ ), X = DMTr, Y = H), which will become a starting material for oligonucleotide synthesis, 25 can be easily obtained by benzylation of Compound 11.



The second method is a method performed via Compound 13 which can be easily obtained from D-ribose in accordance with the literature [3) A.G.M. Barrett et al., J. Org. Chem., 55, 3853 (1990); 4) G.H. Jones et al., *ibid.*, 44, 1309 (1979)]. That is, Compound 13 was led to Compound 16 by three steps, and cyclized under basic conditions to obtain a desired methylglycosyl compound 17. The OMe group at the 1-position of this compound can be substituted by different natural nucleic acid bases or nonnatural nucleic acid base analogues by various methods which have already been developed. For example, a method as shown by a scheme ranging from Compound 17 to Compound 20 can be employed.

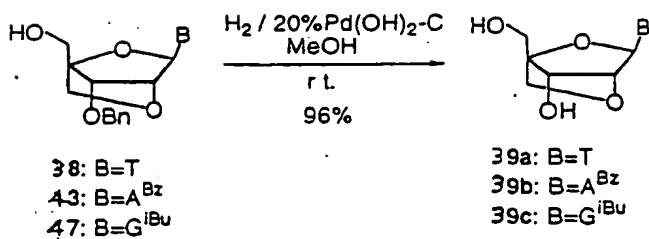
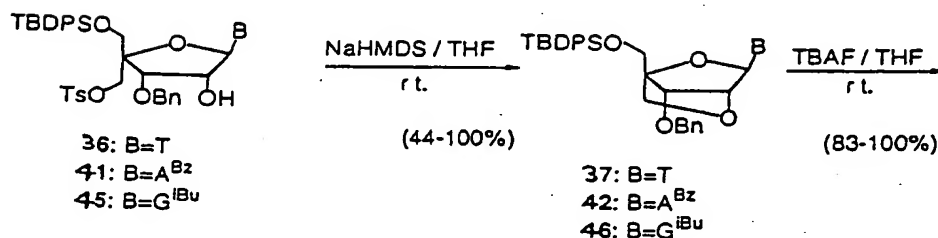
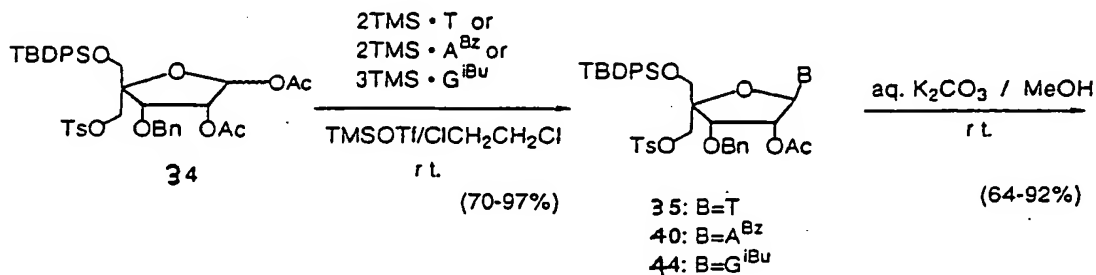


The third method starts with diacetone D-glucose, which is obtained from D-glucose by one step and is commercially available. Compound 31 was prepared in accordance with a reference 5) R.D. Youssefeyeh, J.P.H. Verheyden and J.G. Moffatt., J. Org. Chem., 44, 1301-1309 (1979). Then, Compound 31 was treated as shown by the following scheme to protect the two primary hydroxyl groups with a t-butyldiphenylsilyl group and a p-toluenesulfonyl group progressively. The protected compound was acetylated into Compound 34.



Compound 34 was condensed, separately, with thymine, benzoyladenine, and isobutyrylguanine activated upon trimethylsilylation (referred to as 2TMS·T, 2TMS·A<sup>Bz</sup>, and 3TMS·G<sup>iBu</sup>, respectively), to obtain Compounds 35, 40 and 44 in high yields, as indicated by the scheme offered below.

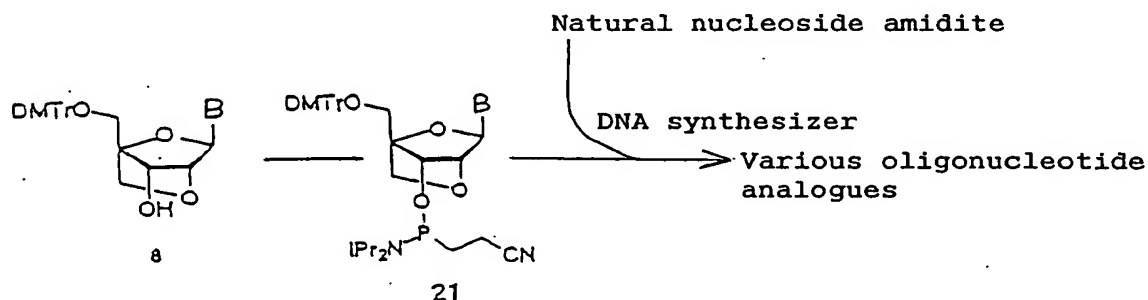
Then, these condensates were subjected to deacetylation (Compounds 36, 41, 45), five-membered ring formation (Compounds 37, 42, 46), desilylation (Compounds 38, 43, 47), and further debenzylation to form desired compounds 39.





(2) Synthesis of oligonucleotide analogue

Compound 8 is reacted with 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphoramidite to obtain an amidite compound 21. This compound is combined with a naturally occurring  
5 nucleoside amidite, and subjected to a DNA synthesizer to synthesize various oligonucleotide analogues. The synthesized crude products are purified using a reversed phase chromatographic column (Oligo-Pak). The purity of the purified product is analyzed by HPLC, whereby the formation  
10 of a purified oligonucleotide analogue can be confirmed.



At least one monomer unit as compound 8 can be contained in the oligonucleotide analogue. Alternatively, the monomer units may be present at two or more locations in the oligonucleotide analogue in such a manner as to be  
15 separated from each other via one or more naturally occurring nucleotides. The present invention makes it possible to synthesize an antisense molecule incorporating a necessary number of the nucleotide analogues (nucleoside analogues) of the invention (a necessary length of the  
20 nucleotide or nucleoside analogue) at a necessary location. The length of the entire oligonucleotide analogue is 2 to 50, preferably 10 to 30, nucleoside units.

Such an oligonucleotide analogue (antisense molecule) is minimally degradable by various nucleases, and can be existent in vivo for a long time after administration. This antisense molecule functions, for example, to form a stable double helix together with a messenger RNA, thereby inhibiting the biosynthesis of a potentially pathogenic protein; or form a triple helix in combination with double-stranded DNA in a genome to inhibit transcription to messenger RNA. The oligonucleotide analogue can also suppress the proliferation of a virus which has infected.

In light of these findings, an oligonucleotide analogue (antisense molecule) using the nucleoside analogue of the present invention is expected to be useful as drugs, including antineoplastics and antivirals, for treatment of diseases by inhibiting the actions of particular genes.

The antisense molecule using the nucleotide (nucleoside) analogue of the present invention can be formulated into parenteral preparations or liposome preparations by incorporating customary auxiliaries such as buffers and/or stabilizers. As preparations for topical application, it may be blended with pharmaceutical carriers in common use to prepare ointments, creams, liquids or plasters.

Synthesis of the nucleoside analogue and nucleotide analogue of the present invention will be described in more detail by way of the following Examples and Production Examples. In these Examples, uracil is mainly used as a base, but other purine nucleic acid bases, pyrimidine

nucleic acid bases and analogues thereof can also be used similarly.

[Example 1] Synthesis of nucleoside analogue

(1) Synthesis of 2',3'-O-cyclohexylidene-4'-(p-toluenesulfonyloxymethyl)uridine (Compound 2)

To an anhydrous pyridine solution (13.5 ml) of Compound 1 (956 mg, 2.70 mmols) known in the literature, p-toluenesulfonyl chloride (771 mg, 4.05 mmols) was added at room temperature in a stream of nitrogen, and the mixture was stirred for 5 hours at 60°C.

To the reaction mixture, a saturated sodium bicarbonate solution was added, whereafter the reaction system was extracted with benzene 3 times. The organic phase was washed once with a saturated sodium chloride solution, and dried over anhydrous  $\text{MgSO}_4$ . The solvents were distilled off under reduced pressure, and the residue was subjected to azeotropy with benzene 3 times. The resulting crude product was purified by silica gel column chromatography ( $\text{CHCl}_3$ :MeOH = 15:1), and then reprecipitated from benzene-hexane to obtain a white powder (Compound 2) (808 mg, 1.59 mmols, 59%).

Compound 2: White powder, m.p. 104-106°C (benzene-hexane). IR  $\nu$  (KBr): 3326, 2929, 2850, 1628, 1577, 1544, 1437, 1311, 1244  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $d_6$ -acetone):  $\delta$  1.45-1.67 (10H, m), 2.45 (3H, s), 3.71 (2H, ABq,  $J = 12$  Hz), 4.20 (2H, ABq,  $J = 11$  Hz), 4.92 (1H, d,  $J' = 6$  Hz), 5.05, 5.06 (1H, dd,  $J = 4.6$  Hz), 5.60 (1H, d,  $J = 7$  Hz), 5.75 (1H, d,  $J = 4$  Hz), 7.48 (2H, d,  $J = 8$  Hz), 7.77 (1H, d,  $J = 8$  Hz), 7.81 (2H, d,

J = 8 Hz), 10.10 (1H, s,). <sup>13</sup>C-NMR (d<sub>6</sub>-acetone): δ 21.5, 24.1, 24.5, 25.5, 34.8, 36.9, 63.5, 69.7, 82.5, 84.7, 87.8, 92.9, 102.9, 115.4, 128.8, 130.8, 133.9, 142.7, 145.9, 151.3, 163.5. Mass (EI): m/z 481 (M<sup>+</sup>-H<sub>2</sub>O).

5            Anal. Calcd. for C<sub>23</sub>H<sub>28</sub>N<sub>2</sub>O<sub>9</sub>S·1/3 H<sub>2</sub>O: C, 53.69; H, 5.61; N, 5.44; S, 6.22. Found: C, 53.99; H, 5.48; N, 5.42; S, 6.10.

(2)        Synthesis of 4'-(p-toluenesulfonyloxymethyl)uridine  
            (Compound 3)

            The above compound 2 (107 mg, 0.21 mmol) was stirred  
10    in TFA-H<sub>2</sub>O (98:2, 1 ml) for 10 minutes at room temperature.  
            The reaction mixture was distilled off under reduced  
            pressure, and EtOH was added to the residue, followed by  
            performing azeotropy 3 times. The resulting crude product  
            was purified by silica gel column chromatography (CHCl<sub>3</sub>:MeOH  
15    = 10:1) to obtain Compound 3 (85.0 mg, 0.20 mmol, 94%).

            Compound 3: White powder, m.p. 119-120°C. IR ν  
            (KBr): 3227, 3060, 2932, 2837, 1709, 1508, 1464, 1252, 978,  
            835, 763, 556 cm<sup>-1</sup>. <sup>1</sup>H-NMR (d<sub>6</sub>-acetone): δ 2.31 (3H, s), 2.84  
            (3H, s), 3.71 (2H, s), 4.13, 4.20 (2H, ABq, J = 11 Hz), 4.28,  
20    4.31 (1H, dd, J' = 9.6 Hz), 4.36 (1H, d, J' = 6 Hz), 5.54  
            (1H, d, J' = 8 Hz), 5.75 (1H, d, J = 7 Hz), 7.32 (2H, d, J =  
            8 Hz), 7.67 (2H, d, J = 8 Hz), 7.70 (1H, d, J' = 8 Hz),  
            10.14 (1H, s). <sup>13</sup>C-NMR (d<sub>6</sub>-acetone): δ 21.5, 63.7, 70.8, 72.7,  
            74.6, 86.8, 88.8, 103.1, 128.8, 130.7, 133.9, 141.7, 145.8,  
25    151.8, 163.9. Mass (EI): m/z 256 (M<sup>+</sup>-OTs).

(3)        Synthesis of 2',3'-O-benzylidene-4'-(p-  
            toluenesulfonyloxymethyl)uridine (Compound 4)

            In a stream of nitrogen, benzaldehyde (2.4 ml,

excess) and zinc chloride (670 mg, 5.0 mmols) were added to the above compound 3 (400 mg, 0.93 mmols), and the mixture was stirred for 5 hours at room temperature. After the reaction was stopped by addition of a saturated sodium bicarbonate solution, the reaction mixture was extracted with chloroform, and washed with a saturated sodium bicarbonate solution, water, and a saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate. The solvents were distilled off under reduced pressure, and the residue was purified by silica gel column chromatography ( $\text{CHCl}_3$ :MeOH = 40:1) to obtain Compound 4 (380 mg, 0.74 mmol, 80%).

Compound 4: White powder. m.p. 99-102°C ( $\text{CH}_2\text{Cl}_2$ -hexane).  $[\alpha]_D^{23}$ -26.7° (c = 1.0,  $\text{CHCl}_3$ ). IR  $\nu$  (KBr): 3059, 1691, 1460, 1362, 1269, 1218, 1177  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  2.41 (3H, s), 3.25 (1H, br), 3.79 (2H, m), 4.19 (2H, s), 5.09 (1H, d, J = 7 Hz), 5.28 (1H, dd, J = 3.7 Hz), 5.60 (1H, d, J = 4 Hz), 5.73 (1H, d, J = 8 Hz), 5.94 (1H, s), 7.24 (1H, d, J = 8 Hz), 7.38 (2H, d, J = 9 Hz), 7.42 (5H, br), 7.69 (2H, d, J = 9 Hz), 9.11 (1H, br).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  21.6, 63.5, 68.3, 77.2, 82.8, 84.2, 87.7, 94.9, 102.6, 107.5, 126.5, 127.9, 128.5, 129.7, 132.2, 135.0, 143.0, 145.0, 150.4, 163.5.

Anal. Calcd. for  $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_9\text{S} \cdot 1/3 \text{H}_2\text{O}$ : C, 55.17; H, 4.76; N, 5.36; S, 6.14. Found: C, 55.19; H, 4.66; N, 5.29; S, 5.98.

(4) Synthesis of 3'-O-benzyl-4'-(p-toluenesulfonyloxymethyl)uridine (Compound 5)

To an acetonitrile solution (3 ml) of Compound 4 (150

mg, 0.29 mmol), sodium borocyanohydride (92 mg, 1.5 mmols) was added at room temperature in a stream of nitrogen. Then, titanium tetrachloride (0.16 ml, 1.5 mmols) was added dropwise under cooling with ice, and the mixture was stirred  
5 for 15 hours at room temperature. The reaction mixture was diluted with chloroform, and washed with a saturated sodium bicarbonate solution, water, and a saturated sodium chloride solution. Then, the organic phase was dried over anhydrous sodium sulfate. After the solvents were distilled off, the  
10 residue was purified by silica gel column chromatography (CHCl<sub>3</sub>:MeOH = 25:1) to obtain Compound 5 (112 mg, 0.22 mmol, 75%).

Compound 5: Colorless crystals. m.p. 195-197°C (AcOEt-hexane).  $[\alpha]_D^{23}$ -14.6° (c = 1.0, CHCl<sub>3</sub>). IR  $\nu$  (KBr):  
15 3033, 2885, 2820, 1726, 1470, 1361, 1274, 1175, 1119 cm<sup>-1</sup>.  
<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 2.40 (3H, s), 3.59-3.77 (3H, m), 4.10, 4.24 (2H, AB, J = 11 Hz), 4.32 (1H, d, J = 6 Hz), 4.56 (2H, m), 4.69 (1H, d, J = 11 Hz), 5.52 (1H, d, J = 6 Hz), 5.67 (1H, d, J = 8 Hz), 7.24-7.29 (7H, m), 7.48 (1H, d, J = 8 Hz),  
20 7.70 (2H, d, J = 9 Hz), 9.91 (1H, s). <sup>13</sup>C-NMR (CDCl<sub>3</sub>):  $\delta$  21.6, 63.2, 69.2, 73.6, 74.6, 78.1, 86.6, 92.9, 102.5, 127.9, 128.2, 128.3, 128.6, 129.9, 132.3, 136.9, 142.4, 145.2, 150.7, 163.8.

Anal. Calcd. for C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>9</sub>S: C, 55.59; H, 5.05; N, 5.40; S, 6.18. Found: C, 55.41; H, 5.02; N, 5.32; S, 6.15.

(5) Synthesis of 3'-O-benzyl-2'-O, 4'-C-methyleneuridine (Compound 6)

To an anhydrous THF solution (1.5 ml) of Compound 5

(80 mg, 0.16 mmol), an anhydrous benzene suspension (0.7 ml) of NaHMDS (3.2 mmols) was added at room temperature in a stream of nitrogen, and the mixture was stirred for 20 hours at room temperature. A saturated sodium bicarbonate solution was added to the reaction mixture, followed by extracting the mixture with  $\text{CHCl}_3$ . The organic phase was washed with a saturated sodium chloride solution, and then dried over anhydrous sodium sulfate. After the solvents were distilled off under reduced pressure, the resulting crude product was purified by silica gel column chromatography ( $\text{CHCl}_3$ :MeOH = 10:1), and then recrystallized from MeOH to obtain Compound 6 (41 mg, 0.10 mmol, 61%).

Compound 6: Colorless crystals. m.p. 217-219°C (MeOH).  $[\alpha]_D^{23} +108.4^\circ$  (c = 0.3, MeOH). IR  $\nu$  (KBr): 3059, 2951, 1688, 1459, 1271, 1053  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $d_6$ -DMSO)  $\delta$ : 3.75, 3.85 (2H, AB, J = 8 Hz), 3.77 (2H, d, J = 5 Hz), 3.92 (1H, s), 4.44 (1H, s), 4.60 (2H, s), 5.39 (1H, t, J = 5 Hz), 5.48 (1H, s), 7.31 (5H, m), 7.72 (1H, d, J = 8 Hz), 11.37 (1H, s).  $^{13}\text{C-NMR}$  ( $d_6$ -DMSO)  $\delta$ : 56.0, 71.1, 71.6, 75.8, 76.5, 86.5, 88.3, 100.9, 127.4, 127.6, 128.2, 137.9, 139.0, 150.0, 163.3. Mass (EI): m/z 346 ( $M^+$ , 1.1).

Anal. Calcd. for  $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_6$ : C, 58.96; H, 5.24; N, 8.09. Found: C, 58.67; H, 5.23; N, 8.05.

(6) Synthesis of 2'-O,4'-C-methyleneuridine (Compound 7)

To a methanol solution (2.5 ml) of Compound 6 (25 mg, 0.072 mmol), 10% Pd-C (25 mg) was added, and the mixture was stirred for 15 hours at atmospheric pressure in a stream of hydrogen. The reaction mixture was filtered, and the

solvent was distilled off. Then, the residue was purified by silica gel column chromatography ( $\text{CHCl}_3$ :MeOH = 10:1, then 5:1) to obtain Compound 7 (18.3 mg, quant.).

Compound 7: Colorless crystals. m.p. 239-243°C (MeOH).  $[\alpha]_D^{23} +92.2^\circ$  (c = 0.3, MeOH). IR  $\nu$  (KBr): 3331, 3091, 3059, 2961, 1689, 1463, 1272, 1049  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$ : 3.76, 3.96 (2H, AB, J = 8 Hz), 3.90 (2H, s), 4.04 (1H, s), 4.28 (1H, s), 5.55 (1H, s), 5.69 (1H, d, J = 8 Hz), 7.88 (1H, d, J = 8 Hz).

Anal. Calcd. for  $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_6$ : C, 46.88; H, 4.72; N, 10.93. Found: C, 46.74; H, 4.70; N, 10.84.

(7) 5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-C-methyleneuridine (Compound 8)

To Compound 7 (140 mg, 0.53 mmol), anhydrous pyridine was added, followed by performing azeotropy of the mixture 3 times. Then, the product was converted into an anhydrous pyridine solution (1.5 ml), and 4,4'-dimethoxytrityl chloride (210 mg, 0.63 mmol) and DMAP (6.5 mg, 0.053 mmol) were added at room temperature in a stream of nitrogen. The mixture was stirred for 5 hours at room temperature. To the reaction mixture, a saturated sodium bicarbonate solution was added, followed by extraction with  $\text{CH}_2\text{Cl}_2$ . The organic phase was washed with water and a saturated sodium chloride solution, and then dried over anhydrous sodium sulfate. After the solvents were distilled off under reduced pressure, the resulting crude product was purified by silica gel column chromatography ( $\text{CHCl}_3$ :MeOH = 40:1) to obtain Compound 8 (230 mg, 0.34 mmol, 66%).



Compound 8: White powder. m.p. 117-120°C (CHCl<sub>3</sub>).

[ $\alpha$ ]<sub>D</sub><sup>23</sup>+17.2° (c = 1.0, CHCl<sub>3</sub>). IR  $\nu$  (KBr): 3393, 3101, 2885, 1689, 1464, 1272, 1047 cm<sup>-1</sup>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 2.59 (1H, br), 3.56 (2H, q, J = 7, 11 Hz), 3.87 (1H, d, J = 7 Hz), 4.26 (1H, s), 4.47 (1H, s), 5.60 (1H, d, J = 9 Hz), 5.63 (1H, s), 5.84 (4H, d, J = 9 Hz), 7.22-7.45 (9H, m), 7.93 (1H, d, J = 9 Hz).

[Example 2] Synthesis of nucleoside analogue

(1) ~~Synthesis of methyl=5-O-(t-butyldiphenylsilyl)-4-hydroxymethyl-2,3-O-isopropylidene- $\beta$ -D-ribofuranoside (Compound 14)~~

In a stream of nitrogen, Et<sub>3</sub>N (2.62 ml, 18.8 mmols) and t-butyldiphenylsilyl chloride (4.88 ml, 18.8 mmols) were added to an anhydrous CH<sub>2</sub>Cl<sub>2</sub> solution (40 ml) of Compound 13 (2.00 g, 8.54 mmols) known in the literature under cooling with ice, and the mixture was stirred for 13 hours at room temperature. To the reaction mixture, a saturated sodium bicarbonate solution was added, whereafter the reaction system was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 5:1) to obtain colorless oily matter (Compound 14) (2.82 g, 5.98 mmols, 70%).

[ $\alpha$ ]<sub>D</sub><sup>17</sup>-16.2° (c = 0.52, CHCl<sub>3</sub>). IR  $\nu$  (KBr): 3510, 3061, 2938, 2852, 1465, 1103 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.09 (9H, s), 1.28 (3H, s), 1.49 (3H, s), 3.22 (3H, s), 3.67, 3.76 (2H, AB, J = 11 Hz), 3.88, 3.93 (2H,

AB,  $J = 11$  Hz), 4.49 (1H, d,  $J = 6$  Hz), 4.57 (1H, d,  $J = 6$  Hz), 4.93 (1H, s), 7.38 - 7.43 (6H, m), 7.67 (4H, d,  $J = 7$  Hz).

$^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta_c$ : 19.2, 24.4, 25.9, 26.9, 55.0, 62.9, 64.8,  
5 82.2, 85.9, 88.7, 108.6, 112.6, 127.8, 129.9, 133.0, 135.7.

Anal. Calcd. for  $\text{C}_{26}\text{H}_{36}\text{O}_6\text{Si} \cdot 1/4 \text{H}_2\text{O}$ : C, 65.45; H, 7.71. Found: C, 65.43; H, 7.59.

(2) Synthesis of methyl-5-O-(*t*-butyldiphenylsilyl)-2,3-O-isopropylidene-4-(*p*-toluenesulfonyloxymethyl)- $\beta$ -ribofuranoside (Compound 15)

In a stream of nitrogen,  $\text{Et}_3\text{N}$  (3.92 g, 28.0 mmols), *p*-toluenesulfonyl chloride (1.34 g, 7.22 mmols), and 4-dimethylaminopyridine (90 mg, 0.72 mmol) were added to an anhydrous  $\text{CH}_2\text{Cl}_2$  solution (15 ml) of Compound 14 (2.13 g,  
15 4.51 mmols), and the mixture was stirred for 17 hours at room temperature. To the reaction mixture, a saturated sodium bicarbonate solution was added, whereafter the reaction system was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium  
20 chloride solution, and then dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 10:1) to obtain colorless oily matter, Compound 15 (2.76 g, 4.42 mmols, 98%).  $[\alpha]_D^{17}$ -  
25  $3.82^\circ$  ( $c = 0.56$ ,  $\text{CHCl}_3$ ). IR  $\nu$  (KBr): 2934, 2852, 1369, 1104  $\text{cm}^{-1}$ .

$^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.02 (9H, s), 1.20 (3H, s), 1.32 (3H, s), 2.41 (3H, s), 3.09 (3H, s), 3.51, 3.77 (2H, AB,  $J = 10$  Hz),

4.34 (1H, d, J = 6 Hz), 4.25, 4.39 (2H, AB, J = 9 Hz), 4.47 (1H, d, J = 6 Hz), 4.77 (1H, s), 7.28, 7.81 (4H, AB, J = 9 Hz), 7.39 - 7.44 (6H, m), 7.62 - 7.65 (4H, m), 7.81 (2H, d, J = 9 Hz).

5  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta_c$ : 19.2, 21.6, 24.5, 25.8, 26.8, 54.9, 62.7, 68.8, 81.9, 85.6, 87.5, 108.7, 112.8, 127.7, 127.8, 128.2, 129.6, 129.9, 132.9, 135.6, 144.4.

Anal. Calcd. for  $\text{C}_{33}\text{H}_{42}\text{O}_8\text{SSi}$ : C, 63.23; H, 6.75; S, 5.11.

Found: C, 62.99; H, 6.53; S, 5.13.

10 (3) Synthesis of methyl=5-O-(t-butyldiphenylsilyl)-4-(p-toluenesulfonyloxymethyl)- $\beta$ -D-ribofuranoside  
(Compound 16)

Trifluoroacetic acid (14 ml) was added to a THF- $\text{H}_2\text{O}$  [11 ml, 8:3 (v/v)] solution of Compound 15 (645 mg, 1.03 mmol) at room temperature, and the mixture was stirred for 20 minutes at room temperature. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 5:1) to obtain colorless oily matter, Compound 16 (464 mg, 0.79 mmol, 77%).  $[\alpha]_D^{17}$ -35.8° (c=1.90,  $\text{CHCl}_3$ ) IR  $\nu$  (KBr): 3499, 3051, 2931, 2840, 1594, 1468, 1362, 1109  $\text{cm}^{-1}$ .

$^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.02(9H, s), 2.42(3H, s), 3.16(3H, s), 3.54, 3.70(2H, AB, J=10Hz), 3.97(1H, d, J=5Hz), 4.18(1H, d, J=5Hz), 4.26, 4.39(2H, AB, J=10Hz), 4.73(1H, s), 7.30(2H, d, J=8Hz), 7.36-7.44 (6H, m), 7.59-7.66(4H, m), 7.78(2H, d, J=8Hz).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta_c$ : 19.2, 21.6, 26.7, 55.2, 66.5, 69.6, 74.0, 75.2, 76.5, 84.8, 107.5, 127.7, 128.0, 129.8, 132.6, 132.7, 132.8, 135.5, 135.6, 144.9.

Anal. Calcd for  $C_{30}H_{38}SSiO_8 \cdot 1/4 H_2O$ : C, 60.94; H, 6.56. Found: C, 60.94; H, 6.43.

5  
MB57

(4) Synthesis of methyl=5-O-(t-butyldiphenylsilyl)-2-O,4-C-methylene- $\beta$ -D-ribofuranoside (Compound 17) and methyl=5-O-(t-butyldiphenylsilyl)-3-O,4-C-methylene- $\beta$ -D-ribofuranoside (Compound 18)

In a stream of nitrogen, a benzene suspension (1.6 ml) of NaHMDS (3.30 mmols) was added to an anhydrous THF solution (4 ml) of Compound 16 (194 mg, 0.33 mmol) at room temperature, and the mixture was stirred for 1 hour at room temperature. After a saturated sodium bicarbonate solution was added to the reaction mixture, the reaction solvents were distilled off, and the residue was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous  $Na_2SO_4$ . The solvent was distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 5:1) to obtain colorless oily matter, Compound 17 (48 mg, 0.116 mmol, 35%) and colorless oily matter, Compound 18 (59 mg, 0.142 mmol, 43%).

Compound 17: IR  $\nu$  (KBr): 3438, 3064, 1103, 1036  $cm^{-1}$ .

$^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 1.08(9H, s), 2.04(1H, br s), 3.39(3H, s), 3.65, 3.98(2H, AB, J=8Hz), 3.95, 4.02(2H, AB, J=12Hz), 4.02(1H, s), 4.30 (1H, s), 4.79(1H, s), 7.38-7.46(6H, m), 7.65-7.69(4H, m).

$^{13}C$ -NMR ( $CDCl_3$ )  $\delta_c$ : 19.2, 26.7, 55.0, 60.7, 71.2, 73.1, 79.9, 85.5, 104.3, 127.8, 129.9, 130.0, 132.9, 135.6, 135.7.

Anal. Calcd for  $C_{23}H_{30}O_5Si \cdot 1/4 H_2O$ : C, 65.68; H, 7.34. Found: C, 65.9

8; H, 7.23.

Compound 18: IR  $\nu$  (KBr): 3456, 3058, 2938, 2852, 1467, 1108  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.10(9H, s), 3.26(3H, s), 3.71(2H, s), 4.02(1H, d,  $J=6\text{Hz}$ ), 4.35, 4.95(2H, d,  $J=7\text{Hz}$ ), 5.01(1H, s), 5.11(1H, d,  $J=6\text{Hz}$ ), 7.38-7.44(6H, m), 7.66(4H, d,  $J=7\text{Hz}$ ).

$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta_c$ : 19.3, 26.8, 55.4, 63.7, 75.1, 77.9, 84.5, 86.3, 111.9, 127.8, 128.0, 129.9, 132.9, 133.0, 135.6, 135.8, 135.9.

Anal. Calcd for  $\text{C}_{23}\text{H}_{30}\text{O}_5\text{Si} \cdot 1/4 \text{H}_2\text{O}$ : C, 65.91; H, 7.34. Found: C, 66.07; H, 7.14.

(5) Synthesis of methyl=3-O-acetyl-5-O-(*t*-butyldiphenylsilyl)-2-O,4-C-methylene- $\beta$ -D-ribofuranoside (Compound 19)

In a stream of nitrogen, acetic anhydride (0.38 ml, 4.08 mmols) and 4-dimethylaminopyridine (21 mg, 0.170 mmols) were added to an anhydrous pyridine solution (10 ml) of Compound 17 (704 mg, 1.70 mmols) at room temperature, and the mixture was stirred for 3 hours at room temperature. After a saturated sodium bicarbonate solution was added to the reaction mixture, the system was extracted with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (hexane:AcOEt = 7:1) to obtain colorless oily matter, Compound 19 (665 mg, 1.46 mmols, 86%).

$[\alpha]_D^{17} -34.3^\circ$  ( $c=0.93, \text{CHCl}_3$ ) IR  $\nu$  (KBr): 3438, 3064, 2934, 1749,

1468, 1103, 1036 $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) $\delta$ : 0.99(9H,s), 1.97(3H,s), 3.34(3H,s), 3.69, 3.86(2H,AB,J=8Hz), 3.86(2H,s), 4.17(1H,s), 4.77(1H,s), 5.06(1H,s), 7.28-7.39(6H,m), 7.58-7.63(4H,m).

5  $^{13}\text{C-NMR}$ ( $\text{CDCl}_3$ ) $\delta_c$ : 19.3, 20.9, 26.7, 55.0, 60.3, 72.0, 73.6, 78.3, 85.3, 104.4, 127.7, 129.8, 133.0, 135.6, 169.8.

Anal. Calcd for  $\text{C}_{25}\text{H}_{32}\text{O}_6\text{Si} \cdot 1/4 \text{H}_2\text{O}$ : C, 65.12; H, 7.10. Found: C, 65.27; H, 7.00.

(6) Synthesis of 5'-O-(t-butyldiphenylsilyl)-2'-O,4'-C-methylene-5-methyluridine (Compound 20)

In a stream of nitrogen, O,O'-bistrimethylsilylthymine (154 mg, 0.598 mmols) was added to an anhydrous  $\text{CH}_3\text{CN}$  solution (2 ml) of Compound 19 (109.2 g, 0.239 mmol) at room temperature. Then, a 1,1-dichloroethane  
15 (0.31 ml) solution of trimethylsilyltrifluoromethane sulfonate (0.82 ml, 8.74 mmols) was added under cooling with ice, and the mixture was stirred for 18 hours at room temperature. The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$ , and a saturated sodium bicarbonate solution was added,  
20 followed by extracting the system with AcOEt 3 times. The organic phase was washed once with a saturated sodium chloride solution, and then dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column  
25 chromatography (hexane:AcOEt = 3:1) to obtain colorless oily matter, Compound 20 (87.7 mg, 0.173 mmol, 70%).

IR  $\nu$  (KBr): 3048, 2935, 2852, 1749, 1466, 1369, 1234, 1108, 1040  $\text{cm}^{-1}$ .

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)δ: 1.06(9H,s), 1.94(3H,s), 2.98(1H,br s), 3.63, 4.00(2H,AB,J=10Hz), 3.72(1H,d,J=7Hz), 3.82-3.84(2H,m), 4.30 (1H,s), 5.25(1H,s), 7.40-7.46(6H,m), 7.60(4H,d,J=6Hz), 7.66 (1H,s), 9.68(1H,br s).

5 [Example 3] Synthesis of nucleoside analogue (different method)

(1) Synthesis of 3-O-benzyl-5-O-t-butyldiphenylsilyl-4-(hydroxymethyl)-1,2-O-isopropylidene-α-D-erythropentofuranose (Compound 32)

10 In a stream of nitrogen, triethylamine (3.71 ml, 26.6 mmols) and t-butyldiphenylsilyl chloride (6.94 ml, 26.7 mmols) were added, under cooling with ice, to a methylene chloride solution (50 ml) of Compound 31 (2.50 g, 8.08 mmols) prepared in accordance with the aforementioned

15 reference 5). The mixture was stirred for 10.5 hours at room temperature. After a saturated sodium bicarbonate solution was added to the reaction mixture, the system was extracted with ethyl acetate. The organic phase was washed with a saturated sodium chloride solution, and then dried

20 over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (AcOEt-hexane: = 1:4 → 1:3) to obtain a white solid, Compound 32 (2.97 g, 5.41 mmols, 67%).

25 m.p. 98-99°C (hexane).  $[\alpha]_D^{20} + 54.8^\circ$  (c = 1.12, acetone).

IR ν max (KBr) : 3553, 2936, 1463, 1379, 1107 cm<sup>-1</sup>.

<sup>1</sup>H-NMR(CDCl<sub>3</sub>)δ: 1.13 (9H, s), 1.50 (3H, s), 1.78 (3H, s), 2.56 (1H, t, J = 7 Hz), 3.82, 3.92 (2H, AB, J = 11 Hz), 3.94 (2H, t,

J = 6 Hz), 4.57 (1H, d, J = 5 Hz), 4.64, 4.95 (2H, AB, J = 12 Hz), 4.83 (1H, dd, J = 4, 5 Hz), 5.95 (1H, d, J = 4 Hz), 7.44-7.55 (11H, m), 7.72-7.78 (4H, m).  $^{13}\text{C-NMR}(\text{CDCl}_3)\delta_c$ : 19.2, 26.2, 26.5, 26.8, 63.2, 65.4, 72.5, 77.9, 79.1, 87.4, 104.4, 113.7, 127.6, 127.7, 128.0, 128.5, 129.5, 129.7, 132.9, 133.1, 134.7, 135.5, 137.2.

Anal. Calcd for  $\text{C}_{32}\text{H}_{40}\text{O}_6\text{Si}$  : C, 70.04; H, 7.38. Found : C, 70.19; H, 7.35.

(2) Synthesis of 3-O-benzyl-5-O-(t-butyldiphenylsilyl)-4-(p-toluenesulfonyloxymethyl)-1,2- $\alpha$ -D-erythropentofuranose (Compound 33)

In a stream of nitrogen, triethylamine (395  $\mu\text{l}$ , 2.83 mmols), p-toluenesulfonyl chloride (139.2 mg, 0.730 mmol), and 4-dimethylaminopyridine (8.92 mg, 0.0730 mmols) were added, under cooling with ice, to a methylene chloride solution of Compound 32 (250 mg, 0.456 mmol). The mixture was stirred for 15.5 hours at room temperature. After a saturated sodium bicarbonate solution was added to the reaction mixture, the system was extracted with ethyl acetate. The organic phase was washed with a saturated sodium chloride solution, and then dried over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (AcOEt-hexane: = 1:6) to obtain light yellow oily matter, Compound 33 (310.6 mg, 0.442 mmol, 97%).  $[\alpha]_D^{20} +16.0^\circ$  (c = 0.44, acetone). IR  $\nu_{\text{max}}$  (KBr) : 2935, 1595, 1462, 1363, 1174, 1106  $\text{cm}^{-1}$ .

$^1\text{H-NMR}(\text{CDCl}_3)\delta$ : 1.08 (9H, s), 1.40 (3H, s), 1.46 (3H, s), 2.48



(3H, s), 3.68, 3.83 (2H, AB, J = 11 Hz), 4.45 (2H, dd, J = 4, 5 Hz), 4.64, 4.81 (2H, AB, J = 12 Hz), 4.68 (1H, dd, J = 4, 5 Hz), 5.81 (1H, d, J = 4 Hz), 7.32 (2H, d, J = 8 Hz), 7.42-7.72 (15H, m), 7.82, (2H, d, J = 8 Hz), 7.66 (4H, m), 7.72 (2H, d, J = 8 Hz).

$^{13}\text{C-NMR}(\text{CDCl}_3)\delta_c$ : 19.1, 21.5, 26.1, 26.4, 26.7, 64.4, 70.0, 72.5, 78.1, 78.9, 85.4, 104.2, 113.6, 127.3, 127.7, 127.9, 128.0, 128.4, 129.6, 129.7, 129.8, 132.7, 132.8, 135.5, 137.2, 144.4. MS(EI) m/z : 646 ( $\text{M}^+ - \text{t-Bu}$ ). High-MS(EI):

10 Calcd for  $\text{C}_{35}\text{H}_{37}\text{O}_8\text{SSi}$  ( $\text{M}^+ - \text{t-Bu}$ ) : 645.1978, Found : 645.1969.

(3) Synthesis of 1,2-di-O-acetyl-3-O-benzyl-5-O-t-butylldiphenylsilyl-4-(p-toluenesulfonyloxymethyl)- $\alpha$ - and - $\beta$ -D-ribofuranose (Compound 34)

In a stream of nitrogen, acetic anhydride (6.0 ml, 63.6 mmols) and concentrated sulfuric acid (56  $\mu\text{l}$ , 1.10  $\mu\text{mol}$ ) were added to an acetic acid solution (56 ml) of Compound 34 (3.70 g, 5.27 mmols). The mixture was stirred for 2 hours at room temperature. The reaction mixture was emptied into iced water (300 ml), and stirred for 30 minutes. After a saturated sodium chloride solution was added, the mixture was extracted with ethyl acetate. Then, the organic phase was dried over magnesium sulfate. The solvents were distilled off, and the resulting crude product was purified by silica gel column chromatography (AcOEt-hexane, 2:1) to obtain yellow oily matter, Compound 34 (3.36 g, 4.53 mmols, 86%), as an  $\alpha$ - $\beta$  (1:4) mixture.

IR  $\nu$  max (KBr) : 2934, 2863, 1751, 1365, 1217, 1106  $\text{cm}^{-1}$ .

$^1\text{H-NMR}(\text{CDCl}_3)$  [ $\beta$ -configuration]  $\delta$ : 1.02 (9H, s), 1.77 (3H, s),

1.98 (3H, s), 2.39 (3H, s), 3.61, 3.76 (2H, AB, J = 11 Hz),  
4.21-4.58 (5H, m), 5.26 (1H, d, J = 5 Hz), 5.94 (1H, s), 7.15-  
7.59 (13H, m), 7.58-7.66 (4H, m), 7.72 (2H, d, J = 8 Hz). [ $\alpha$ -  
configuration] d: 1.02 (9H, s), 1.98 (3H, s), 2.36 (3H, s),  
5 3.48, 3.58 (2H, AB, J = 11 Hz), 4.21-4.58 (5H, m), 5.12 (1H,  
dd, J = 5, 6 Hz), 6.33 (1H, d, J = 5 Hz), 7.15-7.59 (13H, m),  
7.58-7.66 (4H, m), 7.72 (2H, d, J = 8 Hz).

$^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta_c$ : 14.2, 19.3, 20.5, 20.8, 21.6, 26.7, 26.8,  
60.3, 64.8, 69.1, 73.6, 74.1, 78.6, 85.3, 97.4, 127.4, 127.6,  
10 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 129.5,  
129.6, 1289.8, 129.9, 132.4, 132.8, 132.9, 135.4, 135.5,  
135.6, 136.9, 144.5, 168.7, 169.4. High-MS(FAB) :

Calcd for  $\text{C}_{40}\text{H}_{46}\text{N}_2\text{O}_{10}\text{SSiNa}$  ( $\text{M}^+ + \text{Na}$ ) : 769.2479, Found : 769.2484.

(4) Synthesis of 2'-O-acetyl-3'-O-benzyl-5'-O-t-  
15 butyldiphenylsilyl-4'-p-toluenesulfonyloxymethyl-5-  
methyluridine (Compound 35)

In a stream of nitrogen, 2TMS·T (1.04 g, 4.03 mmols)  
and trimethylsilyltrifluoromethane sulfonate (730  $\mu\text{l}$ , 4.03  
mmols) were added, under cooling with ice, to a 1,2-  
20 dichloroethane solution (26 ml) of Compound 34 (1.88 g, 2.52  
mmols), and the mixture was stirred for 17 hours at room  
temperature. A saturated sodium bicarbonate solution was  
added to the reaction mixture, and the system was filtered  
through Celite, followed by extracting the mother liquor  
25 with chloroform. The organic phase was washed with a  
saturated sodium chloride solution, and then dried over  
sodium sulfate. The solvents were distilled off under  
reduced pressure, and the resulting crude product was

purified by silica gel column chromatography (AcOEt-hexane, 2:3) to obtain a white powder, Compound 35 (2.00 g, 2.44 mmols, 97%).

m.p. 70-71.5°C.  $[\alpha]_D^{24} + 4.58^\circ$  (c = 1.25, acetone).

5 IR  $\nu$  max (KBr) : 3059, 2934, 1694, 1465, 1368, 704  $\text{cm}^{-1}$ .

$^1\text{H-NMR}(\text{CDCl}_3)\delta$ : 1.18 (9H, s), 1.63 (3H, d, J = 1 Hz), 2.10 (3H, s), 2.42 (3H, s), 3.73, 3.86 (2H, AB, J = 11 Hz), 4.12, 4.20 (2H, AB, J = 11 Hz), 4.44, 4.57 (2H, AB, J = 11 Hz), 4.45 (1H, d, J = 6 Hz), 5.38 (1H, t, J = 6 Hz), 6.02 (1H, d, J = 6 Hz),  
10 7.21-7.60 (13H, m), 7.62-7.69 (7H, m), 8.91 (1H, br s).

$^{13}\text{C-NMR}(\text{CDCl}_3)\delta_c$ : 11.9, 19.3, 20.6, 21.6, 27.0, 65.3, 68.6, 74.1, 74.8, 77.2, 77.3, 86.0, 86.4, 111.6, 127.9, 128.0, 128.2, 128.5, 129.7, 130.1, 130.2, 131.8, 132.3, 132.5, 135.3, 135.5, 135.6, 136.8, 144.9, 150.2, 163.4, 170.2. MS (FAB) m/z  
15 : 813 ( $\text{M}^+ + \text{H}$ ).

Anal. Calcd for  $\text{C}_{43}\text{H}_{48}\text{N}_2\text{O}_{10}\text{SSi} \cdot 2\text{H}_2\text{O}$ : C, 60.83; H, 6.17; N, 3.30. Found : C, 60.55; H, 5.78; N, 3.22.

(5) Synthesis of 3'-O-benzyl-5'-O-t-butyldiphenylsilyl-4'-p-toluenesulfonyloxymethyl-5-methyluridine  
20 (Compound 36)

Potassium carbonate (12.75 mg, 0.0923 mmol) and water (0.5 ml) were added, under cooling with ice, to a methyl alcohol solution (4 ml) of Compound 35 (250 mg, 0.308 mmol), and the mixture was stirred for 22 hours at room temperature.  
25 Under cooling with ice, acetic acid was added to the reaction mixture to neutralize it, whereafter the solvent was distilled off under reduced pressure. After water was added to the residue, the mixture was extracted with ethyl

acetate. The organic phase was washed with a saturated sodium chloride solution, and then dried over sodium sulfate. The solvent was distilled off under reduced pressure, and then the resulting crude product was purified by silica gel  
5 column chromatography (AcOEt-hexane, 3:2) to obtain a white powder, Compound 36 (216.7 mg, 0.283 mmol, 92%).

mp. 74 - 77°C.  $[\alpha]_D^{23} + 5.15^\circ$  (c = 1.23, CHCl<sub>3</sub>). IR  $\nu$  max (KBr) : 3048, 2934, 1695, 1363, 1181, 1108, 977, 819, 704 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.05 (9H, s), 1.65 (3H, d, J = 1 Hz), 2.39  
10 (3H, s), 3.04 (1H, br d, J = 9 Hz), 3.72 (2H, s), 4.17 (2H, s), 4.18 (1H, d, J = 5 Hz), 4.24-4.32 (1H, m), 4.54, 4.62 (2H, AB, J = 11 Hz), 5.62 (1H, d, J = 6 Hz), 7.19-7.69 (20H, m), 8.46 (1H, br s).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta_c$ : 12.1, 19.4, 26.9, 58.8, 72.0, 72.2, 75.8,  
15 76.7, 87.4, 88.8, 110.4, 127.7, 12.79, 128.1, 128.2, 128.5, 128.7, 129.8, 130.0, 130.1, 132.2, 134.3, 135.3, 135.5, 136.8, 149.8, 163.9. MS(FAB) m/z : 771 (M<sup>+</sup>+H).

Anal. Calcd for C<sub>41</sub>H<sub>46</sub>N<sub>2</sub>O<sub>9</sub>SSi: C, 63.41; H, 6.16; N, 3.51; S, 3.95.

20 Found : C, 63.87; H, 6.01; N, 3.63; S, 4.16.

(6) Synthesis of 3'-O-benzyl-5'-O-t-butyldiphenylsilyl-  
2'-O,4'-C-methylene-5-methyluridine (Compound 37)

In a stream of nitrogen, sodium  
bis(trimethylsilyl)amide (1.0 M in THF, 8.47 ml, 8.47 mmols)  
25 was added, under cooling with ice, to a tetrahydrofuran solution (30 ml) of Compound 36 (1.86 g, 2.42 mmols), and the mixture was stirred for 1 hour at room temperature. A saturated sodium bicarbonate solution (14 ml) was added to

the reaction mixture, and then the solvent was distilled off under reduced pressure. After water was added to the residue, the mixture was extracted with chloroform. The organic phase was washed with a saturated sodium chloride solution, and then dried over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (AcOEt-hexane, 2:3) to obtain a white powder, Compound 37 (1.42 g, 2.37 mmols, 98%).

m.p. 70.5-72°C.  $[\alpha]_D^{22} + 52.47^\circ$  (c = 1.025, acetone). IR  $\nu_{\max}$  (KBr) : 2936, 1694, 1465, 1275, 1106, 1055, 809, 704  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.21 (9H, s), 1.76 (3H, s), 3.88, 4.07 (2H, AB, J = 8 Hz), 4.07, 4.15 (2H, AB, J = 11 Hz), 4.16 (1H, s), 4.66, 4.80 (2H, AB, J = 11 Hz), 4.76 (1H, s), 7.34-7.79 (16H, m), 10.0 (1H, br s). MS (FAB) m/z : 599 ( $\text{M}^+ + \text{H}$ ).

Anal. Calcd for  $\text{C}_{34}\text{H}_{38}\text{N}_2\text{O}_6\text{Si} \cdot 2\text{H}_2\text{O}$ : C, 64.33; H, 6.03; N, 4.41. Found : C, 64.58; H, 6.15; N, 4.28.

(7) Synthesis of 3'-O-benzyl-2'-O,4'-C-methylene-5-methyluridine (Compound 38)

In a stream of nitrogen, tetrabutylammonium fluoride (1.0 M in THF, 379  $\mu\text{l}$ , 0.379  $\mu\text{mol}$ ) was added to a tetrahydrofuran solution (1 ml) of Compound 37 (188.7 mg, 0.316 mmol), and the mixture was stirred for 2.5 hours at room temperature. The reaction mixture was distilled under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (AcOEt-hexane, 1:1  $\rightarrow$  1:0) to obtain a white powder, Compound 38 (94.6 mg, 0.262 mmol, 83%).

IR  $\nu$  max (KBr) : 3424, 3183, 3063, 2950, 1691, 1463, 1273, 1057, 734 $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) $\delta$ : 1.90(3H, d,  $J = 1$  Hz), 3.83, 4.05(2H, AB,  $J = 8$  Hz), 3.93, 4.02(2H, AB,  $J = 12$  Hz), 3.94(1H, s), 4.53(1H, s), 4.56, 4.58(2H, AB,  $J = 12$  Hz), 5.65 (1H, s), 7.32(5H, s), 7.44(1H, d,  $J = 1$  Hz). High-MS (EI):

Calcd for  $\text{C}_{18}\text{H}_{20}\text{NO}_6$  ( $\text{M}^+$ ) : 360.1321, Found : 360.1312.

(8) Synthesis of 2'-O,4'-C-methylene-5-methyluridine  
(Compound 39a)

10 To a methyl alcohol solution (4 ml) of Compound 38 (86.5 mg, 0.240 mmol), 20%  $\text{Pd}(\text{OH})_2\text{-C}$  (86.5 mg) was added, and the mixture was stirred for 14.5 hours at atmospheric pressure in a stream of hydrogen. The reaction mixture was filtered, and then the solvent was distilled off under  
15 reduced pressure to obtain colorless crystals, Compound 39 (62.5 mg, 0.230 mmol, 96%).

mp. 194-195°C.  $[\alpha]_D^{20} + 53.7^\circ$  ( $c = 1.02$ , EtOH). IR  $\nu$  max (KBr) : 3323, 3163, 3027, 2889, 2826, 1689, 1471, 1276, 1057  $\text{cm}^{-1}$ .

20  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$ : 1.89 (3H, q,  $J = 1$  Hz), 3.74, 3.95 (2H, AB,  $J = 8$  Hz), 3.90 (1H, s), 4.07 (1H, s), 4.26 (1H, s), 5.53 (1H, s), 7.74 (1H, d,  $J = 1$  Hz).

$^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$ c: 12.6, 57.6, 70.3, 72.4, 80.8, 88.3, 90.4, 110.7, 136.8, 151.8, 166.5.

25 [Example 4]

(1) Synthesis of 2'-O-acetyl-3'-O-benzyl-5'-O-t-butyl-  
diphenylsilyl-4'-p-toluenesulfonyloxymethyl-N<sup>6</sup>-  
benzoyladenine (Compound 40)

In a stream of nitrogen, a 1,2-dichloroethane solution (5.0 ml) of Compound 34 (250 mg, 0.336 mmol) and trimethylsilyltrifluoromethane sulfonate (6.7  $\mu$ l, 0.0336 mmols) were added, at room temperature, to 2TMS $\cdot$ A<sup>Bz</sup> (128.7 mg, 0.336 mmol) prepared in accordance with a reference 6) (H. Vorbruggen, K. Krolikiewicz and B. Bennua, Chem., Ber., 114, 1234-1255 (1981)). The mixture was heated under reflux for 26 hours. After a saturated sodium bicarbonate solution was added to the reaction mixture, the system was extracted 3 times with methylene chloride. The organic phase was washed with a saturated sodium chloride solution, and then dried over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (CHCl<sub>3</sub>-MeOH, 1:3) to obtain a white powder, Compound 40 (234.5 mg, 0.253 mmol, 75%).

m.p. 77-78°C (AcOEt/hexane).  $[\alpha]_D^{24}$  - 13.2° (c = 1.00, CHCl<sub>3</sub>). IR  $\nu$  max (KBr): 3058, 2934, 1749, 1703, 1606, 1105 cm<sup>-1</sup>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) $\delta$ : 0.99 (9H, s), 2.04 (3H, s), 2.38 (3H, s), 3.74, 3.85 (2H, AB, J = 11 Hz), 4.31, 4.43 (2H, AB, J = 11 Hz), 4.52, 4.58 (2H, AB, J = 11 Hz), 4.81 (1H, d, J = 6 Hz), 5.94 (1H, d, J = 6 Hz), 6.04 (1H, d, J = 5 Hz), 7.18 - 7.61 (20H, m), 7.69 (2H, d, J = 8 Hz), 7.99 (1H, s), 8.01 (2H, d, J = 7 Hz), 8.56 (1H, s), 8.99 (1H, br s). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ c: 19.1, 20.5, 21.5, 26.7, 64.1, 68.4, 74.0, 74.6, 77.9, 86.57, 86.64, 123.4, 127.7, 127.8, 127.9, 128.1, 128.5, 128.8, 129.6, 129.9, 132.0, 132.3, 132.6, 132.7, 133.5, 135.4, 135.5, 136.8, 142.0, 144.7, 149.6, 151.2, 152.6, 164.5, 169.8. MS(FAB) m/z : 926 (M<sup>+</sup>+H).

(2) Synthesis of 3'-O-benzyl-5'-O-t-butyldiphenylsilyl-4'-p-toluenesulfonyloxymethyl-N<sup>6</sup>-benzoyladenosine (Compound 41)

To a methyl alcohol solution (3.0 ml) of Compound 40 (167.9 mg, 0.182 mmol), potassium carbonate (15.0 mg, 0.109 mmol) was added at room temperature, and the mixture was stirred for 15 minute at room temperature. Concentrated hydrochloric acid was added to the reaction mixture to neutralize it, whereafter the system was extracted 3 times with methylene chloride. The organic phase was washed with a saturated sodium chloride solution, and then dried over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (CHCl<sub>3</sub>-MeOH, 30:1) to obtain a white powder, Compound 41 (140.5 mg, 0.160 mmol, 88%).

m.p. 82-83°C (AcOEt-hexane).  $[\alpha]_D^{25}$  - 6.02° (c = 0.96, CHCl<sub>3</sub>).

IR  $\nu$  max (KBr) : 3306, 3066, 2935, 2859, 1701, 1611 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 0.98 (9H, s), 2.37 (3H, s), 3.76 (2H, s), 4.39, 4.45 (1H, AB, J = 11 Hz), 4.54 (1H, d, J = 6 Hz), 4.67, 4.76 (2H, AB, J = 11 Hz), 4.85 (1H, dd, J = 5, 6 Hz), 5.79 (1H, d, J = 5 Hz), 7.20 - 7.58 (21H, m), 7.73 (2H, d, J = 8 Hz), 7.80 (1H, s), 7.96 (2H, d, J = 8 Hz), 8.49 (1H, s), 9.18 (1H, br s).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ c: 19.1, 21.6, 26.8, 64.4, 68.9, 74.1, 74.6, 79.2, 86.8, 89.8, 123.1, 127.7, 127.8, 128.0, 128.2, 128.4, 128.6, 128.8, 129.7, 130.0, 132.1, 132.5, 132.6, 132.8, 133.4, 135.4, 135.5, 136.8, 142.1, 144.8, 149.4, 152.3, 164.5.

(3) Synthesis of 3'-O-benzyl-5'-O-t-butyldiphenylsilyl-



2'-O,4'-C-methylene-N<sup>6</sup>-benzyladenosine (Compound 42)

In a stream of nitrogen, sodium  
bis(trimethylsilyl)amide (1.0 M in THF, 0.58 ml, 0.572 mmol)  
was added to a tetrahydrofuran solution (8.0 ml) of Compound  
5 41 (210.5 mg, 0.238 mmol) at room temperature, and the  
mixture was stirred for 3 hours at room temperature. A  
saturated sodium bicarbonate solution was added to the  
reaction mixture, and then the system was extracted 3 times  
with methylene chloride. The organic phase was washed with  
10 a saturated sodium chloride solution, and then dried over  
sodium sulfate. The solvents were distilled off under  
reduced pressure, and the resulting crude product was  
purified by silica gel column chromatography (CHCl<sub>3</sub>-MeOH,  
30:1) to obtain a white powder, Compound 42 (169.5 mg, 0.238  
15 mmol, quant.).

mp. 80- 81°C. IR  $\nu$  max (KBr): 3259, 3064, 2932, 2858, 1703,  
1607 cm<sup>-1</sup>.

<sup>1</sup>H-NMR(CDCl<sub>3</sub>) $\delta$ : 1.07 (9H, s), 3.95, 4.10 (2H, AB, J = 8 Hz), 4.  
02 (2H, d, J = 8 Hz), 4.56, 4.64 (2H, AB, J = 12 Hz), 4.26 (1H,  
20 s), 4.86 (1H, s), 6.14 (1H, s), 7.26 - 7.70 (18H, m), 8.04 (2  
H, d, J = 7 Hz), 8.22 (1H, s), 8.78 (1H, s), 9.18 (1H, brs).

<sup>13</sup>C-NMR(CDCl<sub>3</sub>)  $\delta$ c: 19.2, 26.5, 26.8, 29.7, 59.2, 72.4, 72.6, 7  
6.5, 76.8, 86.7, 88.6, 123.4, 127.7, 127.8, 127.9, 128.1,  
128.4, 128.8, 129.5, 130.0, 132.4, 132.5, 132.8, 133.5, 134.8,  
25 135.2, 135.5, 135.6, 136.8, 140.4, 152.7.

(4) Synthesis of 3'-O-benzyl-2'-O,4'-C-methylene-N<sup>6</sup>-  
benzoyladenosine (Compound 43)

Tetrabutylammonium fluoride (1.0 M in THF, 1.0 ml,

1.0 mmol) was added, at room temperature, to a tetrahydrofuran solution (7.0 ml) of Compound 42 (173.6 mg, 0.244 mmol), and the mixture was stirred for 25 minutes at room temperature. The reaction mixture was distilled under  
5 reduced pressure, and the resulting crude product was purified by silica gel column chromatography (CHCl<sub>3</sub>-MeOH, 15:1) to obtain a white powder, Compound 43 (115.4 mg, 0.244 mmol, quant.).

mp. 154 - 155°C (Et<sub>2</sub>O). IR  $\nu$  max(KBr): 3339, 2944, 1701, 1611  
10 cm<sup>-1</sup>.

<sup>1</sup>H-NMR(CDCl<sub>3</sub>) $\delta$ : 3.91, 4.13 (2H, AB, J = 8 Hz), 3.93, 4.01 (2H, AB, J = 12 Hz), 4.38 (1H, s), 4.64 (1H, s), 4.85 (1H, s), 6.08 (1H, s), 7.29 (1H, s), 7.51 (2H, d, J = 8 Hz), 7.58 (1H, d, J = 7 Hz), 8.05 (2H, d, J = 7 Hz), 8.14 (1H, s), 8.75 (1H, s), 9.50 (1H, br s).  
15

<sup>13</sup>C-NMR(CDCl<sub>3</sub>) $\delta$ c: 57.1, 72.4, 77.0, 77.1, 86.9, 88.6, 122.9, 127.6, 128.0, 128.1, 128.4, 128.7, 132.8, 133.5, 136.9, 140.5, 149.8, 150.5, 152.8, 165.0.

#### [Example 5]

20 (1) Synthesis of 2'-O-acetyl-3'-O-benzyl-5'-O-t-butyldiphenylsilyl-4'-p-toluenesulfonyloxymethyl-N<sup>2</sup>-isobutyrylguanosine (Compound 44)

In a stream of nitrogen, a 1,2-dichloroethane solution (5.0 ml) of Compound 4 (250 mg, 0.336 mmol) and  
25 trimethylsilyltrifluoromethane sulfonate (6.7  $\mu$ l, 0.0336 mmol) were added, at room temperature, to 3TMS $\cdot$ G<sup>iBu</sup> (146.8 mg, 0.336 mmol) prepared in accordance with the aforementioned reference 6). The mixture was heated under reflux for 15

hours. After a saturated sodium bicarbonate solution was added to the reaction mixture, the system was extracted 3 times with methylene chloride. The organic phase was washed with a saturated sodium chloride solution, and then dried  
5 over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography ( $\text{CHCl}_3$ -MeOH, 30:1) to obtain a white powder, Compound 44 (213.6 mg, 0.235 mmol, 70%).

10 m.p. 96-97°C (AcOEt-hexane).  $[\alpha]_D^{24} -11.09^\circ$  ( $c = 0.97$ ,  $\text{CHCl}_3$ ). IR  $\nu$  max (KBr): 3152, 3065, 2934, 1746, 1681, 1606  $\text{cm}^{-1}$ .

$^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 0.96 (9H, s), 1.10 (3H, d,  $J = 9$  Hz), 1.13 (3H, d,  $J = 9$  Hz), 1.98 (3H, s), 2.36 (3H, s), 2.48 (1H, m),  
15 3.65, 3.72 (2H, AB,  $J = 11$  Hz), 4.23, 4.43 (2H, AB,  $J = 11$  Hz), 4.47 (2H, s), 4.63 (1H, d,  $J = 6$  Hz), 5.74 (1H, t,  $J = 6$  Hz), 5.96 (1H, d,  $J = 6$  Hz), 7.14 - 7.68 (20H, m), 9.15 (1H, s), 12.20 (1H, s).

$^{13}\text{C}$ -NMR( $\text{CDCl}_3$ ) $\delta$ c: 19.1, 19.3, 19.4, 20.8, 21.9, 27.0, 27.2, 36.5, 64.5, 68.9, 74.4, 74.9, 76.7, 86.1, 86.7, 122.0, 127.6, 12  
20 7.7, 127.9, 128.1, 128.3, 128.4, 128.8, 130.1, 130.4, 132.3, 132.7, 132.9, 135.7, 135.8, 137.3, 137.8, 145.2, 147.8, 148.5, 156.2, 170.2, 178.8.

(2) Synthesis of 3'-O-benzyl-5'-O-t-butyldiphenylsilyl-4'-p-toluenesulfonyloxymethyl- $\text{N}^2$ -isobutyrylguanosine  
25 (Compound 45)

To a methyl alcohol solution (3.0 ml) of Compound 44 (137.0 mg, 0.151 mmol), potassium carbonate (15.8 mg, 0.113 mmol) was added at room temperature, and the mixture was

stirred for 45 minutes at room temperature. Concentrated hydrochloric acid was added to the reaction mixture to neutralize it, whereafter the system was extracted 3 times with methylene chloride. The organic phase was washed with  
5 a saturated sodium chloride solution, and then dried over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography ( $\text{CHCl}_3$ -MeOH, 30:1) to obtain a white powder, Compound 45 (83.4 mg, 0.097  
10 mmol, 64%).

mp. 102-103°C (AcOEt-hexane).  $[\alpha]_D^{25} - 2.00^\circ$  ( $c = 0.40$ ,  $\text{CHCl}_3$ ). IR  $\nu_{\text{max}}(\text{KBr})$ : 3166, 2932, 1684, 1607  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 0.90 (9H, s), 1.09 (3H, d,  $J = 7$  Hz), 1.13 (3H, d,  $J = 7$  Hz), 2.30 (1H, m), 2.37 (3H, s), 3.71, 3.76 (2H, AB,  $J = 11$  Hz), 4.32, 4.48 (2H, AB,  $J = 11$  Hz), 4.35 (1H, d,  $J = 6$  Hz), 4.63, 4.90 (2H, AB,  $J = 12$  Hz), 4.96 (1H, t,  $J = 6$  Hz), 5.67 (1H, d,  $J = 7$  Hz), 7.17 - 7.71 (20H, m), 8.82 (1H, s), 12.05 (1H, br s).

$^{13}\text{C-NMR}(\text{CDCl}_3)\delta$ : 18.7, 19.0, 21.6, 26.5, 36.2, 63.5, 69.1, 73.7, 74.3, 78.8, 86.2, 89.5, 127.7, 127.8, 128.0, 128.1, 128.5, 129.7, 130.0, 132.0, 132.6, 132.7, 135.3, 135.4, 137.4, 138.2, 144.8, 146.9, 155.5, 178.5.

(3) Synthesis of 3'-O-benzyl-5'-O-t-butyldiphenylsilyl-2'-O,4'-C-methylene- $\text{N}^2$ -isobutyrylguanosine (Compound  
25 46)

In a stream of nitrogen, sodium bis(trimethylsilyl)amide (1.0 M in THF, 0.31 ml, 0.315 mmol) was added to a tetrahydrofuran solution (3.0 ml) of Compound

45 (92.1 mg, 0.102 mmol) at room temperature, and the mixture was stirred for 3 hours at room temperature. A saturated sodium bicarbonate solution was added to the reaction mixture, and then the system was extracted 3 times  
5 with methylene chloride. The organic phase was washed with a saturated sodium chloride solution, and then dried over sodium sulfate. The solvents were distilled off under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (CHCl<sub>3</sub>-MeOH,  
10 25:1) to obtain a white powder, Compound 46 (31.4 mg, 0.160 mmol, 44%).

mp. 99-100°C. IR  $\nu$  max(KBr): 3162, 3068, 2932, 1683, 1610 cm<sup>-1</sup>.

<sup>1</sup>H-NMR(CDCl<sub>3</sub>) $\delta$ : 1.06 (9H, s), 1.25 (3H, d, J = 7 Hz), 1.27 (3H, d, J = 7 Hz), 2.64 (1H, m), 3.83, 4.01 (2H, AB, J = 8 Hz),  
15 3.97 (2H, d, J = 7 Hz), 4.18 (1H, s), 4.51 (1H, s), 4.54 (2H, d, J = 2 Hz), 5.77 (1H, s), 7.17-7.42 (5H, m), 7.64 - 7.72 (10H, m), 7.84 (1H, s), 9.03 (1H, s), 12.08 (1H, br s).

<sup>13</sup>C-NMR(CDCl<sub>3</sub>) $\delta$ c: 18.9, 19.0, 19.1, 26.5, 26.7, 36.4, 59.1, 72.4, 72.5, 76.8, 77.5, 86.3, 88.3, 121.7, 127.6, 127.7, 127.  
20 8, 127.9, 128.1, 128.4, 129.6, 130.0, 132.36, 132.42, 134.8, 135.45, 135.54, 135.8, 136.8, 146.8, 147.7, 155.4, 178.6.

(4) Synthesis of 3'-O-benzyl-2'-O,4'-C-methylene-N<sup>2</sup>-isobutyrylguanosine (Compound 47)

Tetrabutylammonium fluoride (1.0 M in THF, 0.90 ml,  
25 0.90 mmol) was added, at room temperature, to a tetrahydrofuran solution (3.0 ml) of Compound 46 (41.3 mg, 0.060 mmol), and the mixture was stirred for 1 hour at room temperature. The reaction mixture was distilled under

reduced pressure, and the resulting crude product was purified by silica gel column chromatography (AcOH-EtOH, 20:1) to obtain a white powder, Compound 47 (27.1 mg, 0.060 mmol, quant.).

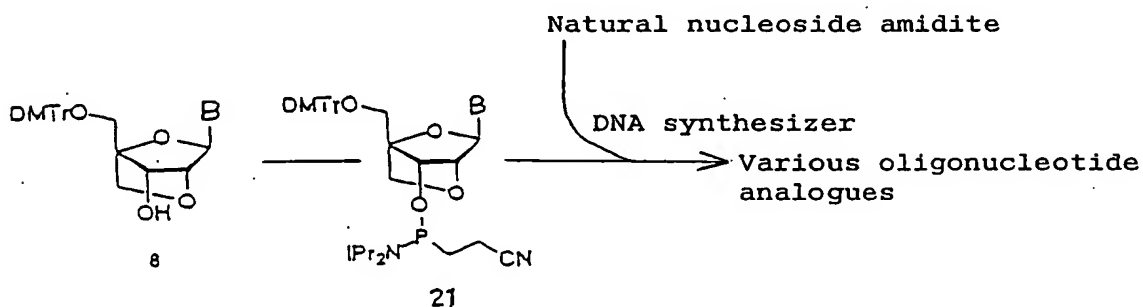
5 mp. 228 - 229°C(Et<sub>2</sub>O).  $[\alpha]_D^{25} + 32.90^\circ$  (c = 0.875, CHCl<sub>3</sub>).

IR  $\nu$  max (KBr) : 3162, 2934, 1683, 1608 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.24 (3H, d, J = 7 Hz), 1.26 (3H, d, J = 7 Hz), 2.76 (1H, m), 3.83, 4.03 (2H, AB, J = 8 Hz), 3.92, 4.02 (2H, AB, J = 13 Hz), 4.33 (1H, s), 4.55 (1H, s), 4.62 (2H, s),  
10 5.80 (1H, s), 7.25 (5H, s), 7.91 (1H, s), 9.85 (1H, s), 12.05 (1H, s).

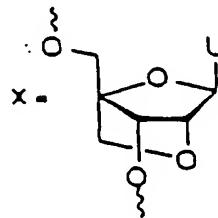
<sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ c: 19.19, 19.25, 36.4, 57.4, 72.5, 77.0, 77.5, 86.5, 88.8, 121.0, 127.8, 128.1, 128.2, 128.3, 128.4, 128.6, 137.1, 137.5, 147.5, 148.2, 155.7, 179.9.

15 [Example 6] Synthesis of oligonucleotide analogue



5'-GCGXTTTTGTCT-3' (XT8)  
5'-GCGTTXTTTGTCT-3' (T2XT3)  
5'-GCGTTTXTTGTCT-3' (T3XT2)  
5'-GCGTTTTTXGCT-3' (T8X)  
5'-GCGXXTTTTGTCT-3' (X2T4)  
5'-GCGTTXXTTGTCT-3' (T2X2T2)  
5'-GCGTTTXXGCT-3' (T4X2)  
5'-GCGXXXXXXGCT-3' (X6)  
5'-GTTTTTTTTTXXC-3' (X2)

*PMB*



(1) 3'-O-[2-cyanoethoxy(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-2'-O,4'-methanouridine  
(Compound 21)

Compound 8 (200 mg, 0.31 mmol) and  
5 diisopropylammonium tetrazolide (39.6 mg, 0.23 mmol) were  
subjected to azeotropy with anhydrous CH<sub>3</sub>CN three times, and  
then the system was converted into an anhydrous CH<sub>3</sub>CN-  
anhydrous THF solution (3:1, 4 ml). In a stream of nitrogen,  
2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite  
10 (0.12 ml, 0.37 mmol) was added, and the mixture was stirred  
for 90 minutes at room temperature. The solvents were  
distilled off under reduced pressure, and the resulting  
crude product was purified by silica gel column  
chromatography (AcOEt:hexane:Et<sub>3</sub>N = 75:25:1). Then, the  
15 purified product was reprecipitated from AcOEt-hexane to  
obtain an amidite compound 21 (181 mg, 0.25 mmol, 81%).  
m.p. 71-74°C (AcOEt-hexane).

<sup>31</sup>P-NMR (CDCl<sub>3</sub>): δ 149.6, 149.5, 149.4, 149.3, 149.2.

(2) General synthesis of oligonucleotide analogues

20 The synthesis of an oligomer was performed by means  
of Pharmacia's DNA synthesizer, Gene Assembler Plus, on a  
0.2 μmol scale. The concentrations of solvents, reagents,  
and phosphoramidite were the same as for the synthesis of  
natural DNA. A DMTr group of 5'-O-DMTr-thymidine (0.2 μmol)  
25 having a 3'-hydroxyl group bound to a CPG support was  
deprotected with trichloroacetic acid. On its 5'-hydroxyl  
group, condensation reaction was repeated using an amidite  
comprising four nucleic acid bases for natural DNA synthesis

and Compound 21 to synthesize oligonucleotide analogues of respective sequences. The synthetic cycle was as follows:

Synthetic cycle (0.2  $\mu$ mol scale)

- 1) Detritylation 1%  $\text{CCl}_3\text{COOH}$  in  $\text{CH}_2\text{ClCH}_2\text{Cl}$ , 6 sec
- 5 2) Coupling 0.1 M phosphoramidite (25 equiv.),  
0.5 M 1H-tetrazole (500 equiv.) in MeCN,  
2 min
- 3) Capping 3% 4-(dimethylamino)pyridine, 10%  $\text{Ac}_2\text{O}$ ,  
in MeCN, 18 sec
- 10 4) Oxidation 0.01 M  $\text{I}_2$  in 2,4,6-collidine/ $\text{H}_2\text{O}$ /MeCN  
(1:5:11), 6 sec

The synthesized oligomer was cleaved from the support by treatment with concentrated aqueous ammonia in the customary manner. At the same time, the protective  
15 cyanoethyl group was detached from the phosphorus atom, and the protective groups for the adenine, guanine and cytosine were also removed.

The resulting 5'-O-dimethoxytritylated oligonucleotide analogue was rid of the DMTr group by use of  
20 5 ml trifluoroacetic acid on a reversed phase chromatographic column (Millipore, Oligo-Pak<sup>TM</sup>SP), and further purified to obtain the desired oligonucleotide analogue.

In accordance with the foregoing method for general  
25 synthesis, the following oligonucleotide analogues were synthesized:

- (2) 5'-GCGXTTTTGCT-3' (XT5)  
Yield 0.06  $\mu$ mol (30% yield)



- (3) 5'-GCGTTXTTTGCT-3' (T2XT3)  
 Yield 0.05  $\mu$ mol (25% yield)
- (4) 5'-GCGTTTXXTTTGCT-3' (T3XT2)  
 Yield 0.03  $\mu$ mol (15% yield)
- 5 (5) 5'-GCGTTTTTXGCT-3' (T5X)  
 Yield 0.06  $\mu$ mol (30% yield)
- (6) 5'-GCGXXTTTTTGCT-3' (X2T4)  
 Yield 0.06  $\mu$ mol (30% yield)
- (7) 5'-GCGTTXXTTTGCT-3' (T2X2T2)  
 Yield 0.05  $\mu$ mol (25% yield)
- (8) 5'-GCGTTTTXXGCT-3' (T4X2)  
 Yield 0.06  $\mu$ mol (30% yield)
- (9) 5'-GCGXXXXXXGCT-3' (X6)  
 Yield 0.06  $\mu$ mol (30% yield)
- 15 (10) 5'-GTTTTTTTTTXXC-3' (X2)  
 Yield 0.07  $\mu$ mol (35% yield)

[Experimental Example 1] Measurement of melting temperature ( $T_m$ )

20 The melting temperatures ( $T_m$ 's) of annealing products between antisense strands, which were the various oligonucleotide analogues synthesized in Example 2, and natural DNA- or RNA-based sense strands were measured to investigate the hybridizing ability of the oligonucleotide

25 analogues of the present invention for complementary DNA and RNA.

Each sample solution (500  $\mu$ L) with end concentrations of 100 mM NaCl, 10 mM sodium phosphate buffer (pH 7.2), 4  $\mu$ M

antisense strand, and 4  $\mu$ M sense strand, respectively, was bathed in boiling water, and slowly cooled to room temperature over the course of 10 hours. The sample solution was gradually cooled to 5°C, kept at 5°C for a  
5 further period of 20 minutes, and then started to be measured, with a stream of nitrogen being passed through a cell chamber of a spectrophotometer (UV-2100PC, Shimadzu) for prevention of moisture condensation. The sample temperature was raised at a rate of 0.2°C/minute until 90°C,  
10 and the ultraviolet absorption at 260 nm was measured at intervals of 0.1°C. To prevent changes in the sample concentration with increases in the temperature, the cell was provided with a closure, and a drop of a mineral oil was applied onto the surface of the sample solution during  
15 measurement.

The results are shown in the following table.

**Table 1** Melting Temperatures (T<sub>m</sub>'s) of Antisense Oligonucleotide Analogues for Complementary DNA and RNA

Antisense molecule	T <sub>m</sub> for complementary DNA <sup>a)</sup> (ΔT <sub>m</sub> /mod.)	T <sub>m</sub> for complementary RNA <sup>b)</sup> (ΔT <sub>m</sub> /mod.)
5' -GCGTTTTTTGCT-3' (natural)	47°C	45°C
5' -GCGXTTTTTGCT-3' (XT5)	50°C (+3°C)	49°C (+4°C)
5' -GCGTTXTTTGCT-3' (T2XT3)	49°C (+2°C)	49°C (+4°C)
5' -GCGTTTXXTTGCT-3' (T3XT2)	49°C (+2°C)	50°C (+5°C)
5' -GCGTTTTTXGCT-3' (T5X)	52°C (+4°C)	51°C (+6°C)
5' -GCGXXTTTTGCT-3' (X2T4)	51°C (+2°C)	53°C (+4°C)
5' -GCGTTXXTTGCT-3' (T2X2T2)	49°C (+1°C)	53°C (+4°C)
5' -GCGTTTTXXGCT-3' (T4X2)	54°C (+3.5°C)	55°C (+5°C)
5' -GCGXXXXXXGCT-3' (X6)	58°C (+1.8°C)	71°C (+4.3°C)

5 a): 3' -CGCAAAAACGA-5'. b): 3' -r(CGCAAAAACGA).

As shown in the table, in the case of the oligomer having one or two units (X) of the nucleoside analogue of the present invention (general formula (Ia)) introduced into  
 10 a natural DNA strand, the ability to hybridize with the

complementary DNA oligomer, evaluated by the  $T_m$ , rose by 2 to 7 degrees (about 2 degrees per modified residue) as compared with the natural strand. With the oligomer having all T's substituted by X's (X6), the increase in the ability  
5 was as high as 11 degrees. When the ability to hybridize with complementary RNA was evaluated, the oligomer incorporating one or two X's had an increase in  $T_m$  of 4 - 10 degrees (4 to 6 degrees per modified residue) over the natural strand. In the case of X6, the ability to hybridize  
10 with complementary RNA was further enhanced, showing an increase in  $T_m$  of more than 25 degrees (4 degrees per modified residue). There have been no examples of analogues undergoing such increases in  $T_m$  as compared with natural strands, and the affinity of the claimed oligomer was higher  
15 for RNA than for DNA. These facts mean that the oligonucleotide analogue composed of the bicyclicoligonucleoside analogue of the present invention has extremely high performance as an antisense molecule, and is useful as a material for pharmaceuticals.

20 [Experimental Example 2] Measurement of nuclease resistance

A buffer solution (0.003 U/ml, 400  $\mu$ l) of a snake venom phosphodiesterase was mixed with a buffer solution (10  $\mu$ M, 400  $\mu$ l) of the oligonucleotide held at 37°C for 15  
25 minutes. The mixed solution was placed in a quartz cell (800  $\mu$ l) kept at 37°C, and increases in the ultraviolet absorption (260 nm) due to the decomposition of the oligonucleotide were measured over time by means of SHIMADZU

UV-2100PC. The buffer used comprised 0.1 M Tris-HCl (pH 8.6), 0.1 M NaCl, and 14 mM MgCl<sub>2</sub>, and was sufficiently degassed before measurement.

Measurement of half-life ( $t_{1/2}$ ):

5            A calculation was made of the average of the values of the UV absorption measured at the start of measurement ( $t=0$ ) and that measured at the time when no increase in this parameter was noted. The time corresponding to this average was designated as the half-life ( $t_{1/2}$ ).

10

Oligonucleotide sequence	$t_{1/2}$ (seconds)
5'-GTTTTTTTTTTC-3' (natural type)	260
5'-GTTTTTTTTT-XX-C-3' (X2)	850

15

Charts showing the time course of the ultraviolet absorption are presented as Fig. 1 (natural strand) and Fig. 2 (X2). The ultraviolet absorption reached a plateau in about 30 minutes for the natural strand, and about 90 minutes for X2, after initiation of the enzyme reaction.

#### INDUSTRIAL APPLICABILITY

The use of this analogue provides an oligonucleotide analogue antisense molecule, which is minimally hydrolyzable with an enzyme in vivo, has a high sense strand binding ability, and is easily synthesized.

Sequence Listing

Name of the applicant: Takeshi Imanishi

Title of the invention: Novel bicyclonucleoside and  
oligonucleotide analogue

5 Reference No.:

Application No.:

Date of application: March , 1998

Priority No.: JPA 53409/97

Priority date: March 7, 1997

10 Number of sequences: 11

Seq. ID No.: 1

Length of sequence: 12

Type of sequence: Nucleotide

15 Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GCGTTTTTTGCT-3'

Seq. ID No.: 2

20 Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GCGXTTTTTGCT-3'

25

Seq. ID No.: 3

Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GCGTTXTTTGCT-3'

5 Seq. ID No.: 4

Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

Number of strands: Single stranded

Topology: Linear

10 Sequence: 5'-GCGTTTXXTTTGCT-3'

Seq. ID No.: 5

Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

15 Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GCGTTTTTXGCT-3'

Seq. ID No.: 6

20 Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GCGXXTTTTTGCT-3'

25

Seq. ID No.: 7

Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GCGTTXXTTGCT-3'

5 Seq. ID No.: 8

Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

Number of strands: Single stranded

Topology: Linear

10 Sequence: 5'-GCGTTTTXXGCT-3'

Seq. ID No.: 9

Length of sequence: 12

Type of sequence: Nucleotide, nucleotide analogue

15 Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GCGXXXXXXGCT-3'

Seq. ID No.: 10

20 Length of sequence: 13

Type of sequence: Nucleotide, nucleotide analogue

Number of strands: Single stranded

Topology: Linear

Sequence: 5'-GTTTTTTTTTTTC-3'

25

Seq. ID No.: 11

Length of sequence: 13

Type of sequence: Nucleotide, nucleotide analogue



**Sequence:** 5'-GTTTTTTTXXTC-3'

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100